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EFFECTS OF ENVIRONMENTAL TEMPERATURE ON THE DEVELOPMENT OF THE MYOTOMAL WHITE MUSCLE IN LARVAL CARP (*CYPRINUS CARPIO* L.)

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Summary

A study was conducted on common carp (*Cyprinus carpio* L.) to determine the effects of environmental temperature experienced by embryos and larvae on the development of myotomal white muscle. Eggs from one female were divided into two groups following fertilisation and incubated at constant pre-hatch temperatures of 18 or 28 °C. At hatching, larvae from the 18 °C-incubated eggs were divided into two groups and either reared at the same temperature of 18 °C ('cold' group) or transferred over a period of 5 days (at 2 °C per day) to 28 °C ('transferred' group). Larvae hatched from eggs incubated at 28 °C were reared at the same temperature of 28 °C ('warm' group). Larvae were sampled at two developmental stages (stage 1, inflation of the back chamber of the swimbladder; stage 2, inflation of the front chamber of the swimbladder) and at 26 days post-hatching.

The maturation of myotome shape during larval life was studied in parallel with the changes occurring in the organisation of white fibres. At stage 1, the epaxial part of the myotomes surrounding the vent had the shape of lamellae inclined backwards, and only one central layer of white fibres was present. At stage 2, the epaxial part of the myotomes began to acquire a V-shape, which was well developed at 26 days post-hatch. At stage 2 and at 26 days post-hatch, two layers of white fibres were identified: the initial central layer and a second apical layer. These differ in their orientation, the initial central layer being orientated backwards and the apical layer forwards, and in the mean fibre diameter, which is greater in the initial central layer.

Studies on the effects of temperature (constant 18 °C, constant 28 °C, transfer from 18 to 28 °C at hatching) were carried out according to both the developmental stage and the length of the larvae. At stage 1, no significant differences were found between the three groups for larval standard length and muscle variables. The number of fibres in one quadrant of epaxial white muscle sectioned at the level of the vent was 100–111. At stage 2, there were significant differences between groups. Larval standard length and mass were higher in the cold group than in the warm group. The transferred larvae were of intermediate standard length but had a significantly higher cross-sectional area of white muscle than either of the other two groups. This increase in surface area was related to a 50 % greater fibre number (233) in the transferred larvae compared with the cold (165) or the warm (152) larvae. The increase in fibre number was more marked for large-diameter (>20 μm) white fibres located in the initial central fibre layer (+58–72 % in transferred larvae) than in small-diameter (⩽10 μm) white fibres mainly located in the apical layer (+18–35 %). In 26 days post-hatch samples, transferred larvae still showed a higher total number of white fibres than warm larvae, but the difference was no longer significant when the total number of white fibres was regressed against larval standard length, suggesting that this stimulation may be temporary.

Key words: fish, *Cyprinus carpio*, larva, temperature, muscle development, myotome, hyperplasia, hypertrophy, white muscle.

Introduction

In fish, muscle tissue forms a larger part of the mass of the body than it does in other vertebrates. In adult fish, locomotor muscle represents approximately 40–60 % of the total body mass. It is subdivided into segmentally arranged myotomes of a complex W-shape, delimited by connective tissue myosepta into which the myotomal muscle fibres insert (Bone, 1978). In the myotomal musculature of many fish, a superficial layer of red fibres covers a main mass of white fibres, the proportion of these two fibre types differing among species (Bone, 1978). In most teleost fish, these two layers of fibres are separated by an intermediate zone, which varies from species to species in both quantity and fibre type composition (Mascarello et al., 1986).

The anatomy of locomotor muscles in the early stages of fish

differs from that observed in the adult. Data on the changes occurring in myotomes during fish muscle development are scarce. In the zebrafish (*Brachydanio rerio*), the initial block shape of the somites is kept until the 12-somite stage. The somites then change to a V-shape over a few hours, at a speed that is a function of the position of the somite along the longitudinal axis of the zebrafish embryo; the adult W-shape is only reached 6 weeks after hatching (Van Raamsdonk et al., 1974). Muscle fibres are originally formed *in ovo* parallel to the body axis and, during the free-living stage, acquire (Van Raamsdonk et al., 1974) the helicoid orientation present in the adult (Alexander, 1969).

Muscle growth occurs both by hypertrophy (fibre enlargement) and by hyperplasia (addition of new fibres). During the normal growth of mammals, the increase in the number of muscle fibres generally stops at or shortly after birth (Goldspink, 1972). In fish, the ability to increase the number of muscle fibres is maintained for longer, even after the juvenile stage (Weatherley et al., 1979). This ability is related to the fish species (Weatherley and Gill, 1984) and, for a given species, to the size of the fish (Weatherley et al., 1980).

Three main phases of hyperplasia occur during fish muscle development: an 'embryonic' phase, giving rise to the fibres present at hatching, a 'larval' phase, consisting mainly of the recruitment of new fibres in proliferative zones located at the surface of the myotome, and a 'juvenile' phase, involving the recruitment of myogenic cells located around previously formed fibres. The 'embryonic' phase of myogenesis involves two populations of muscle precursors that have been distinguished in the segmental plate of the zebrafish embryo: medial (adaxial) cells adjacent to the notochord that express abundant levels of the myogenic regulatory factor MyoD, and lateral (presomitic) cells (Devoto et al., 1996). During segmentation, adaxial cells elongate along the antero-posterior axis until they span the length of the somite; they then migrate radially, becoming a superficial layer of muscle cells that differentiate into slow muscle fibres (Devoto et al., 1996). Lateral presomitic cells remain deep in the myotome and differentiate into fast muscle fibres (Devoto et al., 1996).

At hatching, myotomal fish muscle consists in most species of a main deep white layer and a thin superficial monolayer of embryonic red fibres (for a characterisation of these layers in carp in terms of fibre number, fibre size and histochemical and biochemical properties, see Talesara and Urfi, 1987). Depending on the species, the so-called 'larval' hyperplastic process (i.e. the apposition of new fibres at the edges of the myotomes) was first noted to occur either before hatching (Stickland et al., 1988) or after a variable delay following hatching (Matsuoka and Iwai, 1984; Veggetti et al., 1990, 1993, 1999; Brooks and Johnston, 1993; Gibson and Johnston, 1995; Rowlerson et al., 1995; Stoiber and Sanger; 1996; Alami-Durante et al., 1997; Johnston et al., 1998; Galloway et al., 1999). A study in three European cyprinid species and in rainbow trout (*Oncorhynchus mykiss*) suggested that this superficial growth of white muscle is induced by attachment of presumptive myogenic cells that originate from, and proliferate within, the adjacent mesenchymal tissue lining (Stoiber and Sanger, 1996). The timing of the appearance of the third hyperplastic process (which is referred to as 'juvenile') varies among species. In some fish, it is not detected until the juvenile phase (Matsuoka and Iwai, 1984; Brooks and Johnston, 1993; Brooks et al., 1995; Rowlerson et al., 1995; Veggetti et al., 1999), while in some other species the process begins in larvae (Nag and Nursall, 1972; Veggetti et al., 1990; Stoiber and Sanger, 1996; Alami-Durante et al., 1997; Johnston et al., 1998) and in others it apparently does not occur at all (Veggetti et al., 1993). This 'juvenile hyperplastic process' is attributed either to the *in-situ* proliferation of presumptive deep muscle satellite cells (Koumans and Akster, 1995; Stoiber and Sanger, 1996) or to the migration of the mesenchyme-derived muscle cell precursors that enter the myotomes *via* the myosepta (Stoiber and Sanger, 1996). Both hyperplasia and hypertrophy depend on external factors such as temperature and feeding levels (Weatherley et al., 1979).

Temperature has a great influence on the metabolism, swimming activity, food intake and growth of fish (Brett et al., 1969; Fry, 1971). In juveniles and adults of some fish species such as carp, which have a wide thermal range, changes in temperature regime result in alterations in myofibrillar ATPase activity (Heap et al., 1985), expression of contractile protein isoforms (Crockford and Johnston, 1990; Watabe et al., 1992) and myosin heavy chain gene expression (Gerlach et al., 1990; Goldspink et al., 1992).

In embryos and larvae of fish, development and growth are closely linked to their thermal environment. The speed of embryonic and larval development increases with temperature, thus decreasing the time required to reach a particular stage of development (see Penaz et al., 1983, for carp). Pre-hatch temperature affects the rate of yolk and oil globule utilisation (Heming and Buddington, 1988), larval body size and differentiation at hatching (Blaxter, 1992; Kamler, 1992) and the number of vertebrae of the fish (Fowler, 1970; Niesslbeck et al., 1988; Murray and Beacham, 1989).

The influence of pre-hatch temperature on white muscle cellularity of newly hatched larvae has been demonstrated in species such as Atlantic salmon *Salmo salar* (Stickland et al., 1988; Usher et al., 1994; Nathanailides et al., 1995; Johnston and McLay, 1997), herring *Clupea harengus* (Vieira and Johnston, 1992; Johnston, 1993; Johnston et al., 1995), plaice *Pleuronectes platessa* (Brooks and Johnston, 1993), turbot *Scophthalmus maximus* (Gibson and Johnston, 1995), whitefish *Coregonus lavaretus* (Hanel et al., 1996) and cod *Gadus morhua* (Galloway et al., 1998). However, no general trend is evident for these species. In some species, such as salmon, a decrease in incubation temperature increases the number of deep white fibres present at hatching (e.g. Usher et al., 1994). The reverse is apparently true in other species such as plaice (Brooks and Johnston, 1993). Moreover, for a given species, the response of deep white muscle cellularity to incubation temperature may vary according to the origin of the broodstock. Such differences have been recorded in herring (Vieira and Johnston, 1992; Johnston, 1993) and in salmon

(Usher et al., 1994; Nathanailides et al., 1995; Johnston and McLay, 1997). Inter- and intra-species comparisons of muscle cellularity at hatching are complicated by the fact that, in most cases, it is difficult to separate the differences related to the effects of temperature on hatching size from those related to a direct effect of temperature on fibre hypertrophy and hyperplasia.

Reports of the effects of early manipulations of temperature on the subsequent muscle growth of fish reared thereafter under similar conditions are scarce. In Atlantic salmon reared at very different temperatures up to hatching and then at temperatures that became less and less different up to 150 days post-fertilisation, different numbers of white fibres were recorded when fish were compared at the same stage (first feeding) or at the same body length (2.5 cm) (Nathanailides et al., 1995). In herring reared under different fluctuating temperatures up to first feeding and then transferred to a common fluctuating temperature regime (increasing in a first experiment on Clyde herring, decreasing in a second experiment on Manx herring), different numbers of white muscle fibres were recorded when fish were compared at times of equal growth opportunity after the transfer (80–86 days), i.e. at different body lengths (Johnston et al., 1998).

The aim of the present experiment was to evaluate in a warm-water, fast-growing species, the common carp (*Cyprinus carpio* L.), the extent to which the cellularity of the myotomal white muscle could be modified by temperature manipulations during the embryonic and larval stages (constant low or high temperature from fertilisation to 26 days post-hatching, or transfer from a low to a high temperature at hatching). In this study, the characterisation of the effects of temperature on white muscle cellularity takes into account both the developmental stage and the length of the larvae because the length of carp larvae has been shown to be an important factor in determining white fibre number and width (Alami-Durante et al., 1997).

Materials and methods

Fish and sampling stages

Eggs of *Cyprinus carpio* (L.), obtained by artificial spawning of one female fertilised by two males, were divided into two batches just after fertilisation and incubated at 18 or 28 °C. The eight- to nine-somite stage occurred 15 h post-fertilisation at 28 °C and 34 h post-fertilisation at 18 °C. Hatching started 2 days post-fertilisation at 28 °C and 5 days post-fertilisation at 18 °C.

At hatching, larvae from the 28 °C-incubated eggs were randomly distributed in a rearing system (Charlon and Bergot, 1984) at the same temperature of 28 °C; these will be referred to as 'warm' larvae. The larvae from the 18 °C-incubated eggs were divided into two groups at hatching. One group was distributed in a rearing system at the same temperature of 18 °C (these will be referred to as 'cold' larvae). The other group was transferred to a system in which the temperature was progressively increased from 18 to 28 °C over a period of 5 days (at 2 °C per day, between day 5 and day 9 post-fertilisation) and were kept at 28 °C thereafter (these larvae will be referred to as 'transferred' larvae). Each of the three treatment groups (warm, cold and transferred) consisted of five replicates of 400 larvae each. All the larvae were fed to excess with a microparticulate diet based on liver and yeast and complemented with 8 % vitamins, 5 % minerals and 2 % cod liver oil (Alami-Durante et al., 1997) provided during a 14 h daily light period (08:00–22:00 h) from the first day after the beginning of hatching.

Two developmental stages were sampled per temperature group: (i) the inflation of the back chamber of the swimbladder, which will be referred to as 'stage 1' and which, in carp reared at 25 °C, occurs a few hours before the first exogenous feeding (Penaz et al., 1983); and (ii) the inflation of the front chamber of the swimbladder, which will be referred to as 'stage 2'. These stages were chosen because they are easily identified in semi-transparent larvae. Stage 1 occurred 1 day post-hatch (3 days post-fertilisation) in the warm group and 2 days post-hatch (7 days post-fertilisation) in the other two groups, i.e. only 2 days after transfer and at a temperature of 22 °C for the transferred group. Stage 2 occurred 5 days post-hatch (7 days post-fertilisation) in the warm group, and 20 days post-hatch (25 days post-fertilisation) in the cold group. The corresponding age was 8 days post-hatch (13 days post-fertilisation) in the transferred group, i.e. 3 days after the end of the temperature rise. Additional samples were also taken 26 days post-hatch (D26) from the three groups, i.e. 28 days post-fertilisation in the warm group and 31 days post-fertilisation in the other groups.

Sampling involved removing 3–4 larvae from each of the five replicates of a temperature treatment. The larvae were pooled to produce a total of 15 larvae per temperature treatment and stage. After being killed by an overdose of anaesthetic (2-phenoxy-ethanol), all larvae were fixed and dehydrated as described previously (Alami-Durante, 1990) before individual lengths (total length, standard length), height (body height at the level of the vent) and mass measurement were made. The coefficient of condition, *K*, was chosen as a morphological index of the larvae. It was calculated as $100W/SL^3$, where *W* is the larval wet mass (mg) and *SL* is the standard length (mm) of the larvae.

Muscle histology

Larvae were paraffin-embedded, cut transversely into sections 10 μm thick and stained with haematoxylin and Orange G according to Gabe (1968). Shape and cellular analyses of white muscle were performed on an epaxial quadrant of white muscle, as defined previously (Alami-Durante et al., 1997).

To reconstruct the longitudinal inclination of the dorsal part of the myotomes, ribbons of serial transverse sections surrounding the vent were analysed. For each section, outlines of the myosepta visible in an epaxial quadrant of white muscle were drawn. The middle points of these myosepta were then estimated and recorded on a vertical plane (Fig. 1A). The

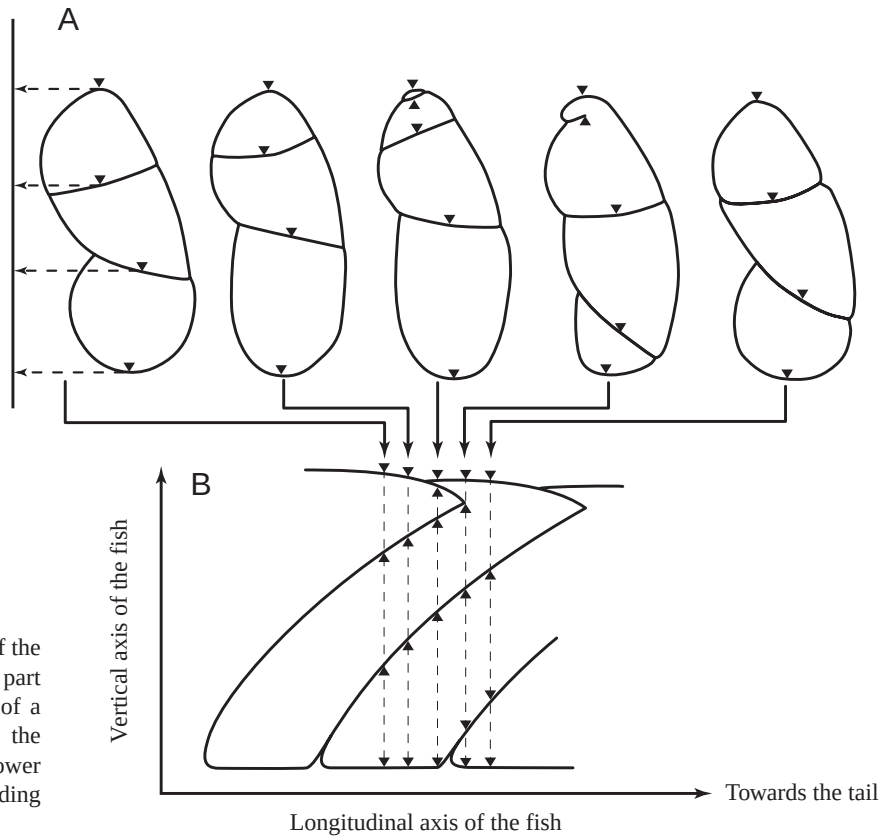


Fig. 1. Principle of the reconstitution of the shape of the dorsal part of myotomes in carp larvae. The upper part of the figure (A) shows serial transverse sections of a quadrant of epaxial white muscle (▼ indicates the estimated middle points of the myosepta). The lower part of the figure (B) shows the corresponding longitudinal reconstruction of myotome shape.

planes corresponding to the different serial sections were then juxtaposed, with an interval corresponding to the thickness of the sections between them. The successive middle points of each myoseptum were then joined by a line. The figure obtained thus corresponded to the calculated longitudinal section of the dorsal part of the myotomes that surrounded the vent (Fig. 1B). Reconstruction of the inclination of the myotomes was undertaken for a small number of larvae only: one cold larva at stage 1, two larvae from each of the cold, transferred and warm batches at stage 2, and one warm larva at D26.

Ribbons of serial transverse sections were also used for the determination of white muscle fibre trajectories in one transferred larva at stage 2. Photographs of serial sections of this larva were taken. Fibres located at different positions in the myotome were identified and their contours on successive sections were drawn on tracing paper. Thereafter, the successive contours of each fibre were stacked to restore its trajectory in successive planes and to calculate the angles it formed with the longitudinal and vertical axes of the larva.

To study muscle cellularity, one transverse section located at the level of the vent was examined per fish. Measurements were made with a semi-automatic image analyser (VIDS, Systèmes Analytiques, France). The total cross-sectional area of one epaxial quadrant of white muscle was measured. In addition, the height and maximum width of the quadrant were measured as parameters of shape. Individual outlines of all the white fibres were drawn, so that the individual fibre cross-sectional area, the individual fibre diameter (the diameter of a circle whose area is the same as that of the muscle fibre), the maximal fibre diameter, the total fibre cross-sectional area (the sum of individual cross-sectional areas) and the total number of fibres could be determined.

Statistical analyses

The results are expressed as mean ± S.D. Individual values of larval length, larval height, larval mass, muscle cross-sectional area, muscle height and width, fibre number, fibre area and fibre diameter of the cold, transferred and warm groups were compared, after logarithmic transformation, by analysis of variance (ANOVA) and Newman–Keuls tests performed using STATITCF software (ITCF, 1988). Analyses of covariance were undertaken, after logarithmic transformation of the data, according to Snedecor and Cochran (1971).

Results

Larval size

At stage 1, no significant differences in total length ($F_{2,24}$=1.22; P=0.312) or standard length ($F_{2,24}$=1.28; P=0.297) were found between the three groups of larvae (Table 1). In contrast, at stage 2, significant differences in mass ($F_{2,24}$=8.77; P=0.001), total length ($F_{2,24}$=16.35; P<0.001), standard length ($F_{2,24}$=20.88; P<0.001), body height ($F_{2,24}$=7.99; P=0.002) and coefficient of condition ($F_{2,24}$=3.50; P=0.045) were evident. The mass, total length,

Table 1. *A comparison of the effects of different temperature regimes, at two developmental stages and at 26 days post-hatching, on the size and shape of carp larvae*

Batch	Sample	*N*	Mass (mg)	Total length (mm)	Standard length (mm)	Body height (mm)	Coefficient of condition, $K=100W/SL^3$ (mg mm^{-3})
Cold	Stage 1	9	ND	6.09±0.26	5.76±0.21	ND	ND
Transferred	Stage 1	9	ND	6.20±0.14	5.89±0.15	ND	ND
Warm	Stage 1	9	ND	6.04±0.21	5.76±0.23	ND	ND
Cold	Stage 2	9	11.3±4.4^a	10.98±0.80^a	9.87±0.67^a	1.19±0.26^a	1.12±0.25^b
Transferred	Stage 2	9	10.7±2.1^a	10.52±0.25^a	9.31±0.21^b	1.04±0.11^a	1.32±0.23^a
Warm	Stage 2	9	6.6±1.2^b	9.66±0.31^b	8.54±0.34^c	0.87±0.10^b	1.06±0.20^b
Cold	D26	5	41±9^b	15.32±0.82^b	13.06±0.54^c	1.78±0.20^b	1.83±0.22^c
Transferred	D26	5	692±166^a	35.29±3.31^a	28.54±2.13^a	6.24±0.68^a	2.92±0.17^b
Warm	D26	5	555±135^a	31.60±2.94^a	25.31±2.19^b	5.58±0.56^a	3.36±0.28^a

For each variable studied at each sampling point, significant differences between batches of larvae ($P<0.05$) are indicated by different letters (analysis of variance and Newman–Keuls test).

N is the number of individuals, *W* is the mass of the larvae, *SL* is the standard length of the larvae.

ND, not determined.

Values are means ± S.D.

standard length and body height of the warm larvae were all significantly lower than those of the larvae from the other two groups. The standard length of the cold larvae was also significantly greater than that of the transferred larvae. The transferred larvae, which were intermediate between the cold and warm groups in terms of standard length, exhibited a significantly higher coefficient of condition (*K*) than the other two groups. At D26, significant differences in mass ($F_{2,12}=177.28$; $P<0.001$), total length ($F_{2,12}=142.24$; $P<0.001$), standard length ($F_{2,12}=168.10$; $P<0.001$), body height ($F_{2,12}=122.58$; $P<0.001$) and coefficient of condition ($F_{2,12}=56.98$; $P<0.001$) were also evident: the larvae of the transferred group had a greater standard length but a lower coefficient of condition than those of the warm group.

Organisation of myotomes

At stage 1, two or three discrete areas of muscle belonging to different myotomes were present in a transverse section of a quadrant of epaxial white muscle of carp larvae. Longitudinal reconstruction of the dorsal part of the myotomes showed that they had the shape of lamellae (a in Fig. 2A) inclined from an antero-ventral position to a postero-dorsal one. These lamellae formed a mean angle of 45° with the longitudinal axis of the fish. Each lamella extended approximately 270 μm with a height of approximately 175 μm and a maximal width of approximately 100 μm. At this stage, irrespective of the temperature group, there was no apparent difference in the size of the white fibres in different locations in the quadrant.

At stage 2, one quadrant of carp epaxial white muscle was made up of 3–5 discrete areas of muscle that were not necessarily from different myotomes. In four out of six larvae, two of the discrete areas of muscle belonged to the same myotome (Table 2). When reconstructing the longitudinal section of the fish, it could be seen that this was due to the

Table 2. *Effects of temperature on the shape of the myotomes of carp larvae at stage 2*

	Cold group		Transferred group		Warm group	
	Larva 1	Larva 2	Larva 1	Larva 2	Larva 1	Larva 2
Larvae						
Standard length (mm)	10.4	9.6	9.0	9.3	8.5	8.7
Myotomes						
Total length (μm)	500	511	387	560	480	428
Total height (μm)	368	296	338	400	315	272
Angle*	45	40	46	41	41	42
Apical layer of the myotome						
Length (μm)	75	No AL	45	77	34	No AL
Angle*	12	No AL	21	14	18	No AL

*Angle (in degrees) with the longitudinal axis of the larva.

No AL, the apical layer of the myotome was non-existent in this larva.

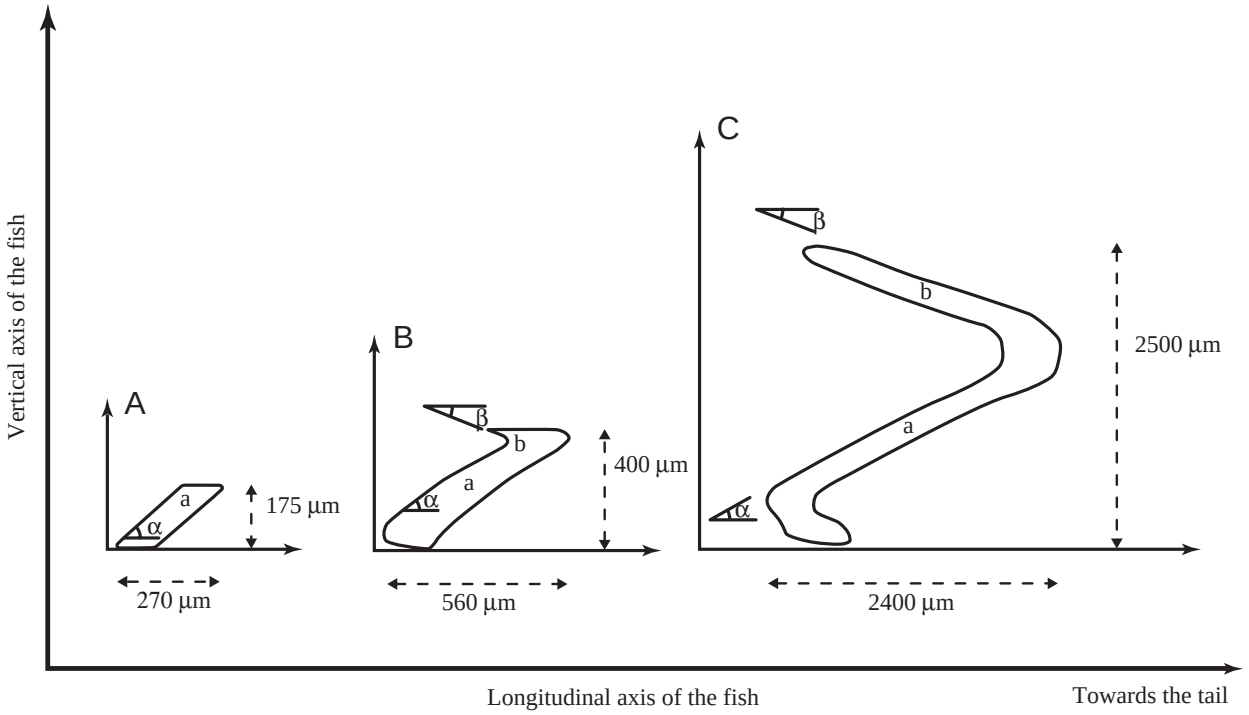
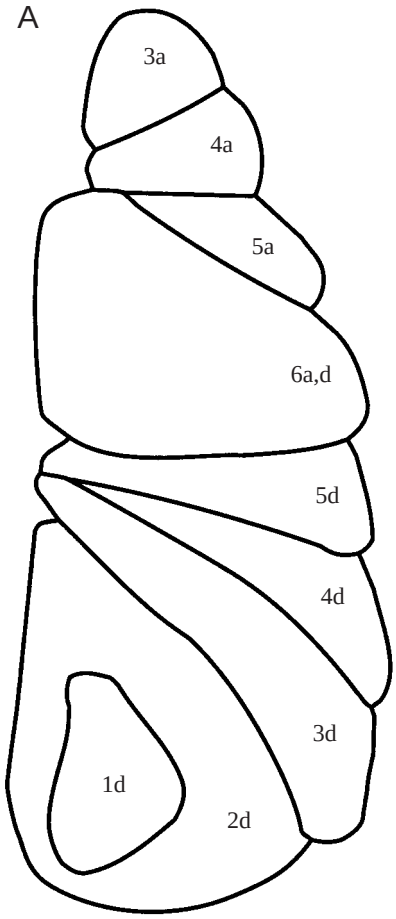


Fig. 2. Reconstruction of a longitudinal section of the dorsal part of a myotome in carp larvae: (A) at stage 1 (inflation of the back chamber of the swimbladder), (B) at stage 2 (inflation of the front chamber of the swimbladder) and (C) at 26 days post-hatching (D26). α, angle between the central layer of white fibres (a) and the longitudinal axis of the larva; β, angle between the apical layer of white fibres (b) and the longitudinal axis of the larva.

dorsal tip of a myotome (b in Fig. 2B) folding forwards. In such cases, the distribution of fibre sizes was not homogeneous through the muscle section. In the part of the section corresponding to the dorsal tip of the myotome that folded forwards (this part will be referred to as apical), all the fibres appeared small in cross-sectional area. Serial sections were examined to confirm that the small fibres located in the apical part were differentiated fibres, running from one side of the myotome to the other. In the rest of the section (which will be referred to as central), the fibres were heterogeneous in size. A study of the trajectories in successive transverse sections of fibres located in this central part of the white muscle indicated that, according to their location, the axes of these fibres made an angle of approximately 3° to approximately 10° with the longitudinal and vertical axes of the larva. The ratio of the total dorsal myotomal length (µm) to larval standard length (mm) was 51–53 in all three groups of larvae, suggesting that the total length of one dorsal myotome at the level of the vent was proportional to the standard length of the larva, irrespective of the temperature. In contrast, the ratio of total dorsal myotomal height (µm) to larval standard length (mm) suggested an effect of temperature transfer on the morphology of the larvae. Indeed, this last ratio was greater in the transferred larvae (40) than in the larvae of the two other batches (33–34), which is consistent with the higher value of *K* of the transferred larvae (Table 1).

At D26, a transverse section through the epaxial part of carp myotomes revealed that nine areas of white muscle separated by myosepta are present (Fig. 3). A longitudinal reconstruction of the fish (Fig. 2C) indicated that these areas belonged to six different myotomes, which are identified by numbers 1–6 in Fig. 3. For each myotome, two areas can be distinguished in longitudinal section (designated a and b in Fig. 2C): the first area (a) consists of the central layer already present at stage 1; the second area (b) corresponds to the apical layer observed at stage 2 but absent from stage 1. The apical layer increased considerably in size during development: at D26, its length (2100 µm) and height (1000 µm) were smaller but of the same order of magnitude as those (length 2400 µm; height 1500 µm) of the initial central layer. The angle formed by the apical part of the myotome (b) and the longitudinal axis of the fish was 20° and that formed by the central part of the myotome (a) and the longitudinal axis of the fish was 28°. Fig. 2C also shows that the lower part of the central layer protruded forwards. This protrusion has the shape of a cone and, in transverse section, appears as a circular zone surrounded by the neighbouring myotome (Fig. 3A). The difference in fibre size distributions between the central layer and the apical layer was smaller at 26 days post-hatching than at stage 2. The two layers of white fibres both contained small and large fibres, but large fibres were more numerous in the central layer, as indicated in Fig. 3B. Mean fibre diameter was lowest in the upper part of the apical layer, greatest in the central part of the central layer and not significantly different between the apex and the base of the cone in the lower part of the central layer (Fig. 3B).

A



B

Myotome section		Number of fibres	Mean diameter of fibres (μm)	Number of fibres	
Number	Layer			⩽14 μm	>40 μm
3	Apical	247	24.3±6.4^{x}	17	0
4	Apical	278	27.2±7.2^{y}	8	2
5	Apical	161	27.7±8.7^{y}	8	4
6	Apical + deep	512	27.7±11.8^{y,z}	62	42
5	Deep	277	29.0±11.0^{y,z}	21	22
4	Deep	177	30.0±11.1^{z}	12	17
3	Deep	294	28.9±9.9^{z,y}	15	16
2	Deep	280	29.0±7.5^{z,y}	4	4
1	Deep	168	27.5±7.1^{y}	2	3
Total		2355		150	110

Fig. 3. (A) Transverse section through a quadrant of epaxial white muscle of a D26 'warm' carp larva and (B) corresponding muscle constituents. The different areas of muscle present in a section are identified by numbers (1–6) according to the myotome to which they belonged and according to their position (a, apical; d, deep) in the myotome. For each area of muscle, the total number of fibres, the mean diameter of the fibres (± S.D.) and the number of fibres with a diameter ⩽14 μm or ⩾40 μm are indicated. Different exponents (x, y, z) represent significant differences (P<0.05) between parts of myotomes.

Effects of temperature treatments on white muscle

At stage 1, no significant differences in total cross-sectional area ($F_{2,24}$=0.01; P=0.986), maximal height ($F_{2,24}$=0.39; P=0.682) or maximal width ($F_{2,24}$=0.20; P=0.823) of white muscle were found between groups (Table 3). The total cross-sectional area of white muscle was approximately 6600 μm^2, its maximal height approximately 150 μm and its maximal width approximately 46 μm. No significant differences were obtained concerning the total cross-sectional area of white fibres ($F_{2,24}$=0.22; P=0.807), the total number of white fibres ($F_{2,24}$=1.86; P=0.177), the mean white fibre cross-sectional area ($F_{2,24}$=0.215; P=0.808), the mean white fibre diameter ($F_{2,24}$=0.294; P=0.748) and the maximal white fibre diameter ($F_{2,24}$=1.278; P=0.297) of the three groups of stage 1 larvae. The total number of white fibres was 100–111, the mean white fibre cross-sectional area was 52 μm^2, the mean white fibre diameter was 7.8–8.0 μm and the maximal white fibre diameter was 13.3–14.3 μm. The shape of the distribution of the white fibre diameters was also similar at this stage for the three groups of larvae (Fig. 4A).

At stage 2, significant differences in total cross-sectional area of white muscle ($F_{2,23}$=15.51; P<0.001), maximal height of white muscle ($F_{2,23}$=18.39; P<0.001), maximal width of white muscle ($F_{2,23}$=6.82; P<0.005), total cross-sectional area of white fibres ($F_{2,24}$=10.92; P<0.001), total number of white fibres ($F_{2,24}$=10.66; P<0.001) and maximal white fibre diameter ($F_{2,24}$=20.88; P<0.001) were evident between the three groups of larvae (Table 3). Cold larvae, which had a greater standard length and body height than warm larvae (Table 1), also exhibited significantly higher values for white muscle height and for maximal fibre diameter (Table 3).

Between stage 1 and stage 2, the total white fibre cross-sectional area increased approximately fivefold in both cold and warm larvae, and approximately eightfold in transferred larvae. This difference was linked to a greater increase in fibre number in the transferred larvae (2.1-fold) than in the larvae kept at constant temperature (1.6-fold) and not to a difference in the increase in mean fibre area (3.4-fold in all three batches of larvae). At stage 2, the fibre number was approximately 50 % higher in the transferred larvae (233±32) than in the cold (165±18) or warm (152±39) larvae.

At stage 2, different distributions of fibre diameters were observed between cold, transferred and warm larvae (Fig. 4B). Separate comparisons (Table 4) using analyses of variance and Newman–Keuls tests of arbitrary classes of diameter (small ⩽10 μm, medium 10–20 μm and large >20 μm) indicated that the proportion of small ($F_{2,24}$=4.62; P=0.020), medium ($F_{2,24}$=10.02; P<0.001) and large ($F_{2,24}$=7.62; P=0.003) fibres was very different between batches of larvae. Simple calculations from Table 4 indicated that the advantage, in term of fibre number, of the transferred larvae over the two other groups was more important for fibres greater than 20 μm in diameter (+58–72 % in transferred larvae) than for fibres less than 10 μm in diameter (+18–35 %). Fig. 5 shows the distributions of white fibre diameters in the two areas of the myotome (the initial central layer and the apical layer; see

Table 3. *Effects of different temperature patterns, compared at two developmental stages and at 26 days post-hatching on the white muscle cross-sectional area, on the white muscle maximal height and maximal width, on the total cross-sectional area of white fibres, on the total number of white fibres, on the mean white fibre cross-sectional area, on the mean white fibre diameter and on the maximal white fibre diameter*

			Whole white muscle			White fibres				
Batch	Sample	*N*	Total cross-sectional area×10^{-3} (μm^2)	Height (mm)	Width (mm)	Total cross-sectional area×10^{-3} (μm^2)	Total number	Mean cross-sectional area (μm^2)	Mean diameter (μm)	Maximal diameter (μm)
Cold	Stage 1	9	6.6±1.8	0.16±0.02	0.04±0.01	5.5±1.6	100±17	52±9	8.0±0.6	14.3±2.3
Transferred	Stage 1	9	6.5±1.3	0.15±0.02	0.05±0.01	5.7±1.3	111±12	52±17	7.8±1.2	14.1±2.0
Warm	Stage 1	9	6.6±1.2	0.15±0.01	0.05±0.01	5.2±1.1	100± 9	52±9	7.8±0.6	13.3±1.6
Cold	Stage 2	9	37.4±12.3[b]	0.32±0.05[b]	0.13±0.03[b]	28.9±7.9[b]	165±18[b]	174±34	13.6±1.3	32.3±3.5[a]
Transferred	Stage 2	9	52.6±8.4[a]	0.38±0.02[a]	0.16±0.02[a]	44.0±8.3[a]	233±32[a]	188±18	13.9±0.7	32.2±1.9[a]
Warm	Stage 2	9	29.3±8.3[b]	0.25±0.05[c]	0.12±0.03[b]	25.3±7.4[b]	152±39[b]	165±25	13.1±1.2	27.7±1.3[b]
Cold	D26	5	158±35	0.59±0.09	0.31±0.02	ND	337±68	ND	ND	ND
Transferred	D26	5	2359±615	2.53±0.44	0.98±0.12	1642±271	2545±327[a]	644±51	27.1±0.4	73.3±3.7
Warm	D26	5	1922±325	2.31±0.36	0.90±0.12	1379±113	2110±200[b]	655±17	26.8±1.1	69.2±6.3

For each variable studied at each sampling point, significant differences between batches of larvae ($P<0.05$) are indicated by different letters (analysis of variance and Newman–Keuls test).

N is the number of individuals.

ND, not determined.

Values are means ± S.D.

Fig. 2) in subsamples of larvae. Large-diameter (>20 μm) white fibres were present only in the initial central layer. Small-diameter (⩽10 μm) white fibres were mostly (85–95 % of their total number) found in the apical layer.

At 26 days after hatching, the cold larvae were no longer comparable with the transferred and warm larvae. Indeed, the cold larvae were approximately 15 times lighter than and half as long as the transferred and warm larvae. In the D26 sample, no significant differences were recorded between the total cross-sectional area of white muscle ($F_{1,8}=1.67$; $P=0.234$), the maximal height of white muscle ($F_{1,8}=0.71$; $P=0.423$), the maximal width of white muscle ($F_{1,8}=1.11$; $P=0.324$), the total cross-sectional area of white fibres ($F_{1,8}=4.07$; $P=0.078$), the mean cross-sectional area of white fibres ($F_{1,8}=0.24$; $P=0.634$), the mean white fibre diameter ($F_{1,8}=0.23$; $P=0.646$) and the maximal white fibre diameter ($F_{1,8}=5.12$; $P=0.054$) of transferred and warm carp larvae, but the total number of white fibres was significantly higher ($F_{1,8}=6.62$; $P=0.033$) in transferred larvae than in warm ones (Table 3). The distributions of the white fibre diameters of transferred and warm larvae had a similar shape (Fig. 4C). An analysis of covariance of the total number of white fibres and the larval standard length (this later variable was taken as the explicative one) indicated that there was no significant difference in the slope ($F_{1,6}=0.03$) or elevation ($F_{1,7}=0.84$) of the regression lines corresponding to the two groups of larvae. For the two groups of larvae, the allometric relationship between the total number of white fibres (*TNF*) and the larval standard length (*SL*) was: $\log_e TNF=3.542+1.277\log_e SL$ ($r^2=0.800$; $N=10$). The difference between transferred and warm carp larvae in the total number of white fibres present 26 days post-hatching was therefore linked to their difference in standard length.

Table 4. *Effects of temperature on the number of white fibres with a small (⩽10 μm), medium (>10 and ⩽20 μm) and large (>20 μm) diameter in carp larvae at stage 2*

	Cold batch	Transferred batch	Warm batch
Small fibres	74.3±17.9[a,b]	87.6±12.8[a]	64.7±16.9[b]
Medium fibres	59.9±15.6[b]	97.9±27.0[a]	59.8±18.1[b]
Large fibres	30.3±13.0[b]	48.0±12.7[a]	27.9±10.0[b]

Values on the same line not sharing a common letter are significantly different at $P<0.05$ (analysis of variance and Newman–Keuls tests).

Values are means ± S.D., $N=9$ larvae per temperature.

Discussion

Development of carp embryos and larvae

The rate of development of carp embryos and larvae observed here at 28 °C was consistent with the values given for the same species by Penaz et al. (1983), who reported that, at 26.7 °C, the 10-somite stage was reached 16 h, hatching 40 h and first swimbladder inflation (stage 1) 77 h after fertilisation. For the 18 °C group, our data are consistent with those obtained at 17.4 °C by Penaz et al. (1983) only up to hatching. Indeed, according to Penaz et al. (1983), the 10-somite stage was reached 35 h and hatching 110 h after fertilisation at 17.4 °C. In the present study, at 18 °C, stage 1 was reached 168 h after

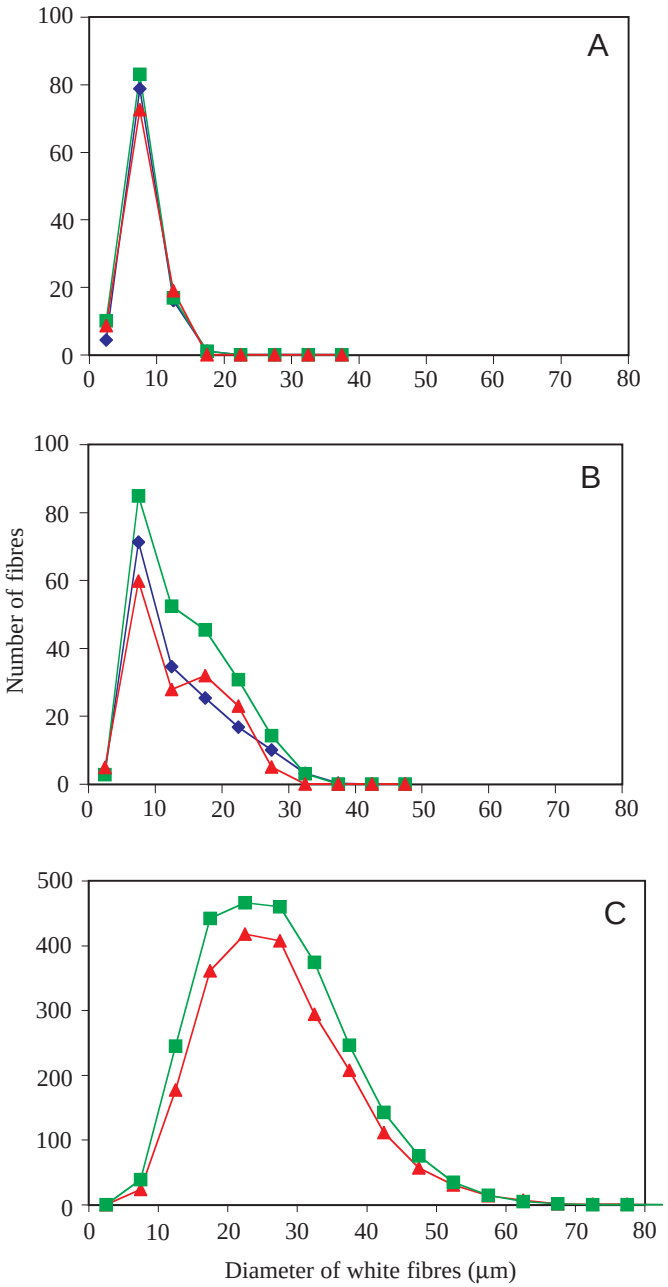


Fig. 4. Distributions of white fibre diameters in a whole white muscle epaxial quadrant of carp larvae at (A) stage 1 (means of nine larvae per group), (B) stage 2 (means of nine larvae per group) and (C) at 26 days post-hatching (D26) (means of five larvae per group). Blue ◆, 'cold' group; green ■, 'transferred' group; red ▲ 'warm' group.

fertilisation, which is intermediate between the time (266 h post-fertilisation) reported by Penaz et al. (1983) at 17.4 °C and that previously observed (150 h post-fertilisation) at 19 °C (H. Alami-Durante, unpublished results). As reported for the Atlantic salmon (*Salmo salar*; Nathanailides et al., 1995) and for the whitefish (*Coregonus lavaretus*; Escaffre et al., 1995), incubation at a low temperature and subsequent rearing of larvae at a higher temperature resulted in rapid growth in carp

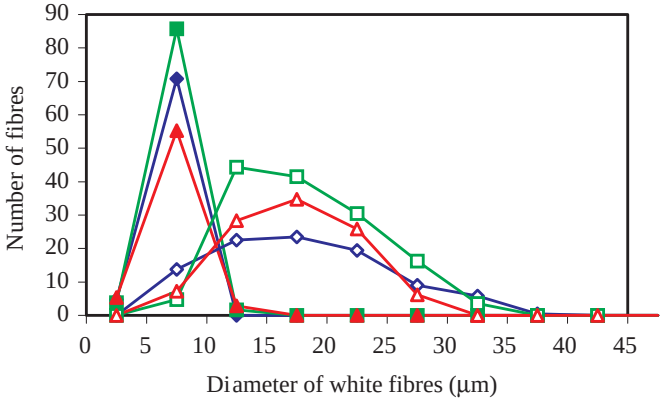


Fig. 5. Distributions of white fibre diameter in the apical layer (filled symbols) and in the central layer (open symbols) of white muscle in subsamples of four cold (blue diamonds), six transferred (green squares) and six warm (red triangles) carp larvae at stage 2.

larvae, with final length and mass being greater than, or similar to, those of larvae kept continuously at high temperature. This could be linked, at least in part, to an improved utilisation of endogenous reserves at low incubation temperature (Escaffre et al., 1995).

Shape of carp larvae

Previous results, demonstrating that the shape of carp larvae changes during the first 3 weeks of feeding (Durante, 1986), are confirmed in the present study. Between stage 2 and D26, the body height of transferred and warm larvae increased much more (approximately sixfold) than their standard length (approximately threefold). Analysis of the coefficient of condition (*K*) of the larvae indicated the existence of some temperature-dependent shape differences. As the transferred larvae were intermediate in standard length and tended to be intermediate in body height between the two other groups of larvae at stage 2, they might also be expected to be intermediate in terms of coefficient of condition. This was not the case. At stage 2, the transferred larvae had a significantly higher *K* value than either of the other groups of larvae. This greater 'roundness' of the transferred carp larvae at stage 2 was linked to a greater quantity of muscle, as demonstrated by a greater total cross-sectional area of white muscle in these larvae.

Development-related changes in myotome shape and white muscle cellularity

In stage 1 carp larvae, the dorsal part of the myotomes had the shape of lamellae inclined towards the tail, as described in 2-day-old zebrafish *Brachydanio rerio* (Van Raamsdonk et al., 1974). However, the mean angle formed by the myotome and the longitudinal axis of 2-day-old larvae was 45° in carp compared with only 36° in zebrafish.

The folding of the upper part of the myotome that occurred in stage 2 carp larvae has also been observed in 3-week-old zebrafish reared at 22 °C (Van Raamsdonk et al., 1974). In carp, this change of myotome shape is due to the addition of a

new layer of fibres on the upper part of the initial central layer present at stage 1. In stage 2 carp larvae, this newly formed apical layer consists largely of small fibres (≤10 μm diameter) and has, in transverse section, the aspect of a proliferative zone, corresponding to the 'germinal' zones described in the course of the development of *Dicentrarchus labrax* (Veggetti et al., 1990), *Pleuronectes platessa* (Brooks and Johnston, 1993), *Scophthalmus maximus* (Gibson and Johnston, 1995), *Sparus aurata* (Rowlerson et al., 1995) and *Gadus morhua* (Galloway et al., 1999). In carp, this apposition of superficial fibres had not begun at first feeding (stage 1) but was well developed 8 days later (Alami-Durante et al., 1997). In some other cyprinids (*Rutilus rutilus*, *Chalcalburnus chalcoides mento*, *Rutilus frisii meidingeri*), the accumulation of small new fibres at the dorsal tip of the myotomes also occurred after the onset of exogenous feeding (Stoiber and Sanger, 1996). The present observations show that the apical zone corresponds to the appearance of a second layer of white fibres, distinct from the initial central layer of white fibres. In this apical layer, the oldest fibres are younger than the biggest (also assumed to be the oldest) fibres of the initial central layer. As early as stage 2, the white muscle fibres constituting the central part of the myotome of carp larvae follow non-linear trajectories, indicating that the adult helicoid orientation of white muscle fibres described in adults of *Poecilia reticulata* (Van der Stelt, 1968), *Xiphophorus maculatus* (Alexander, 1969) and *Brachydanio rerio* (Mos et al., 1988) starts to develop early in the life of fish. However, the angles formed by the fibre trajectories and the axis of the larva (longitudinal and vertical) are small, suggesting that the eventual overestimation of the cross-sectional area of fibres due to their inclination is also low.

In D26 carp larvae, small white fibres occurred throughout the myotome, as described in 28-day-old sea bass (*Dicentrarchus labrax*; Veggetti et al., 1990), but with a variable density depending on the myotome section. At this age, the later-forming apical layer of white fibres was of the same order of size as the central layer of white fibres. These two layers participated in the formation of the posterior upper cone described by Alexander (1969) and Van Raamsdonk et al. (1974). In contrast, the anterior cone described by these authors appeared to consist only of the deep layer, which shows a protrusion of its central part, with no addition of a third fibre layer. Thus, in a 30 mm long carp larva, the shape of the dorsal myotomes is close to the shape of those of a typical adult teleost. The number of myotomes (six) present in a transverse section through the epaxial musculature of a D26 carp larva (30 mm long) is close to that observed (seven) in 35 mm long salmon (Le Danois, 1958).

Length-related changes in white muscle cellularity

To clarify the length-related changes in fibre number occurring in carp larvae, the present data were plotted with previous data (Alami-Durante et al., 1997) obtained from larvae reared in the same system with the same diet. These larvae had been incubated at 21 °C and reared at 26 °C after hatching, or incubated at 15 °C, 19 °C or 23 °C and reared at 23 °C after hatching; they will be referred to as T21, T15, T19 and T23 larvae, respectively. Fig. 6 shows that, from a standard

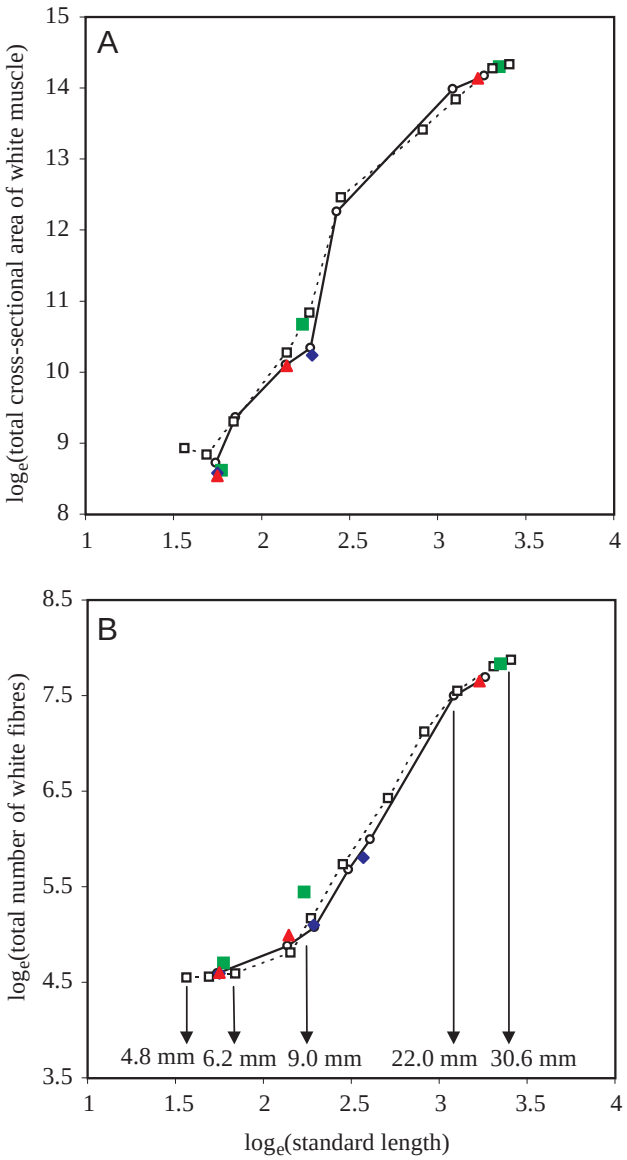


Fig. 6. Pattern of change in (A) the total cross-sectional area (μm^2) of white muscle and (B) the total number of white fibres as a function of the standard length (*SL*, mm) of the carp larvae. Larvae from this study and a previous one (Alami-Durante et al., 1997) were divided into one group of larvae maintained at constant temperature during the embryonic and larval stages ('cold', 'warm', 'T23') and a second group of larvae reared at a higher post-hatch temperature than the pre-hatch one ('transferred', 'T15', 'T19', 'T21'). For each group, individuals were sorted according to their standard length and separated into groups of increasing $0.2\log_e SL$. Mean values of standard length and total fibre number corresponding to these classes are reported, without error bars to simplify the figure. The dotted line joins the means values corresponding to all the transferred larvae (□), while the solid line joins the mean values corresponding to all the larvae maintained at constant temperatures (○). Mean values corresponding to the present experiment are presented as green ■, 'transferred'; blue ◆, 'cold'; and red ▲, 'warm'.

length of 4.8 mm (hatching) up to a standard length of 31 mm, the increase in total white muscle cross-sectional area (*Ac*, μm^2) and in total number of white fibres (*TNF*) as a function of carp standard length (*SL*, mm) occurred in three periods, two periods of slow increase separated by one of faster increase, with slope changes at approximately 9 mm and 22 mm. A similar increase in the total number of white fibres over three periods (two periods of slow increase separated by a period of faster increase) as a function of larval length was found by Fukuda (1990) in Pacific herring. In this species, slope changes were recorded at standard lengths of 15.4 mm and 26.9 mm.

During the first period of carp growth, between a standard length (*SL*) of 4.8 mm and 9.0 mm, the white muscle cross-sectional area increased 4.74-fold, while the total number of white fibres increased only 1.39-fold: 1.04-fold between 4.8 mm *SL* (hatching) and 6.2 mm *SL* (first swimbladder inflation) and 1.33-fold between 6.2 and 9.0 mm *SL*. This indicates that, in carp larvae, the initial muscle mass increase is due to hypertrophy of the white fibres present at hatching, and that hyperplasia begins after first feeding. Similarly, in some other species such as red sea bream (*Chrysophrys major*), Atlantic herring (*Clupea harengus*) and cod (*Gadus morhua*), the total number of white fibres does not increase immediately after hatching but only when the larvae reach a standard length of 3.5–4 mm (sea bream), a total length of 14 mm (Atlantic herring) and a standard length of 5 mm (cod) (Matsuoka and Iwai, 1984; Johnston et al., 1998; Galloway et al., 1999).

In carp larvae 9 mm long, as the second inflation of the swimbladder occurred, the apical layer of white fibres appeared at the dorsal edge of the myotome. During the second period of carp growth, i.e. between standard lengths of approximately 9.0 and 22.0 mm, the total cross-sectional area of white muscle increased 23-fold, while the total number of white fibres increased 9.4-fold. The transition period starting at 9 mm corresponds to a large increase in the size of the apical layer of white fibres. During this period, other important events associated with the locomotor capacity of carp larvae occur: the ratio of total length to standard length and the ratio of mass to $(\text{standard length})^3$ change (Bergot et al., 1989) and the caudal fin rays, hypural plates and vertebrae ossify (Osse and Van den Boogaart, 1995). The modification of swimming performance found by Wakeling et al. (1999) results from all the morphological changes occurring during this period.

During the third period of carp growth, i.e. between standard lengths of approximately 22.0 and 30.6 mm, the total cross-sectional area of white muscle increased 2.5-fold, while the total number of white fibres increased 2.1-fold. The standard length at which a slowing down in the recruitment of fibres was observed (approximately 22 mm) corresponds to a shift in the allometric coefficient between the body depth and the standard length of carp larvae (Osse and Van den Boogaart, 1995) and to the beginning of the juvenile period of development of the carp, characterised by the attainment of a body shape similar to that of adult fish (Penaz et al., 1983).

Effects of pre-hatch temperature on the white muscle cellularity of yolk-sac carp larvae

According to present and previous data (Alami-Durante et al., 1997), when early carp larvae are compared at an equivalent length between hatching and first feeding, the total cross-sectional area of their white muscle and the total number of 'embryonic' white fibres are not significantly affected by constant incubation temperature. The different constant pre-hatch temperatures that were used in this (18 and 28 °C) and previous (15, 19 and 23 °C) studies have not significantly modified the balance between the recruitment of new fibres and fibre growth during the first step of myogenesis occurring in embryonic development. This demonstrates the great ability of the embryos of this species to adapt to environmental temperature and to maintain their length-related programme of increase in white fibre number and size. None of the other fish species studied thus far can be regarded as equally eurythermal.

In flatfish (turbot, plaice), changes in pre-hatch temperature modify the total cross-sectional area of white muscle in newly hatched larvae. In turbot (*Scophthalmus maximus*), incubation at 12 °C instead of 16 °C produces larvae that, 1 day after hatching, are approximately 11 % longer and have a lower mean cross-sectional area of white fibres but the same number of white fibres (Gibson and Johnston, 1995) as 16 °C larvae. In plaice (*Pleuronectes platessa*), incubation at 8 °C instead of 12 °C produces 1-day-old larvae with the same total length but with a smaller fibre number and a smaller fibre cross-sectional area (Brooks and Johnston, 1993).

In some other species (salmon, cod), the total cross-sectional area of white muscle recorded in early larvae was conserved after incubation at different temperatures, but the relative contributions of hyperplasia and hypertrophy were modified. In salmon (*Salmo salar*), incubation at ambient temperature (fluctuating around 1.6 °C) instead of 10 °C produces alevins that, at hatching, are equivalent in length and have the same total cross-sectional area of white muscle, but significantly more white fibres (Stickland et al., 1988). In cod (*Gadus morhua*), incubation at 1 °C instead of 5–8 °C produces larvae that, 1 day after hatching, are shorter but have a similar total cross-sectional area of white muscle and significantly more white fibres (Galloway et al., 1998).

In herring (*Clupea harengus*), different responses to pre-hatch temperature were recorded. First, incubation at 5 °C instead of 10 °C was reported to produce, 1 day after hatching, larvae with a similar length but with a higher mean cross-sectional area of white fibres and a lower number of white fibres (Viera and Johnston, 1992). Second, incubation at 5 °C instead of 8–12 °C was reported to produce larvae that showed a body length in the same range at hatching but with a lower mean cross-sectional area of white fibres, the total number of white fibres being unaffected (Johnston, 1993). These intra-specific differences in the response of white muscle cellularity to pre-hatch temperature may be linked to the fact that herring eggs were obtained from different broodstocks, caught from the wild. The quantity and quality of yolk reserves of embryos

produced by such wild females would not be the same, causing differences in the endogenous potential of embryos for growth.

Effects of temperature on the development of white muscle cellularity in free-swimming carp larvae

The present study indicates that the second step of myogenesis occurring in carp larvae (i.e. the addition of new fibres in the apical zone of the myotome) may be considerably influenced by temperature. Within the different temperature patterns tested in the present and previous experiments, the only one that significantly modified the total number of white fibres in carp larvae, compared at identical standard length, was the progressive transfer temperature pattern tested in the present study (Fig. 6). Nevertheless, its positive effect on the total number of white fibres was not recorded throughout the period sampled. The lack of effect of transfer at stage 1 (i.e. at a standard length of 5.8 mm, only 2 days after the beginning of the transfer and after a temperature rise of only 4 °C) is probably because the temperature change was too short or too small. The higher fibre number recorded in the transferred group was only significant at stage 2 (8.5–9.9 mm standard length), i.e. 3 days after the end of the transfer procedure. At this stage, the standard length of transferred carp larvae was intermediate between those of warm and cold carp larvae. Hence, the variables describing muscle cellularity (white fibre number, white fibre diameter) would be intermediate if muscular development followed the same development pattern irrespective of temperature. This hypothesis is not supported by the present results indicating that, at stage 2, the transferred group of carp larvae had a higher white fibre number at intermediate standard length. In salmon, Nathanailides et al. (1995) reported that alevins reared under a regime of increasing temperature from 3 to 11 °C, instead of at a constant temperature of 11 °C, exhibited a higher white fibre number at a similar fork length (2.5 cm). In herring, an effect of the thermal experience of the embryo on post-larval white muscle growth was also found by Johnston et al. (1998), but fish were compared at times of equal growth opportunity after the transfer (days) and not at equal length.

At stage 2, the effect of the temperature transfer is greater on the number of large-size white fibres (+58–72 %), which at this stage are located only in the central layer, than on the number of recently formed white fibres (+18–35 %), which are essentially located, at this stage, in the apical layer. This indicates that the main effect of the temperature transfer occurs before stage 2, during the temperature rise. At stage 2, i.e. 3 days after the end of the transfer, its positive effect on the total white fibre number is already declining. This positive effect thereafter continued to decline until it became undetectable, in D26 carp larvae, when the total number of white fibres was regressed against larval standard length.

It is also possible to analyse temperature-dependent changes in muscle cellularity by comparing the total number of white muscle fibres occurring in larvae that have the same total cross-sectional area of fibres. Such an analysis was undertaken on the warm, transferred and cold larvae in the present experiment as well as on larvae incubated at 21 °C and reared at 26 °C in a previous study (Alami-Durante et al., 1997). Fig. 7 indicates that there was a clear linear relationship between total white fibre number (*TNF*) and total cross-sectional area of white fibres (*tA*, μm^2) for total cross-sectional areas of white fibres ranging between approximately 11 600 and 2 032 500 μm^2. Analysis of covariance indicated that the relationship between the total number of white fibres and the total cross-sectional area of white fibres was not significantly different for the four groups of larvae. For all these larvae, the allometric relationship between the total number of white fibres (*TNF*) and the total cross-sectional area of white fibres (*tA*, μm^2) was: $\log_e TNF = -1.711 + 0.666 \log_e tA$ (r^2=0.99; N=46; P<0.001).

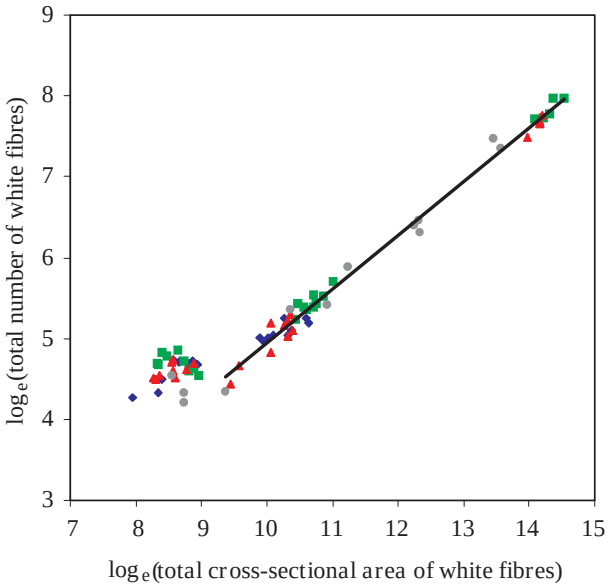


Fig. 7. Increase in the total number of white fibres (*TNF*) as a function of total cross-sectional area of white fibres (*tA*, μm^2). Green ■ 'transferred', blue ◆ 'cold', red ▲, 'warm' carp larvae from the present experiment; grey ● 'T21', values for carp larvae from a previous study (Alami-Durante et al., 1997). For total cross-sectional areas of white fibres ranging between approximately 11 600 μm^2 and 2 032 500 μm^2, the allometric relationship between the total number of white fibres and *tA* was, for the four groups of larvae: $\log_e TNF = -1.711 + 0.666 \log_e tA$ (r^2=0.99; N=46; P<0.001).

The temperature transfer, as conducted in the present study, increased the number of fibres normally present at a given stage and standard length (stage 2; 8.5–9.9 mm *SL*), but did not change the general pattern of increase in white fibre number as a function of the total cross-sectional area of white fibres. Muscle growth of transferred carp larvae thus appeared to have been speeded up only temporarily relative to the length increase of the larvae. According to the present results, the burst of muscle growth in carp larvae associated with a temperature transfer from 18 to 28 °C over 5 days after hatching was temporary. It would be interesting to determine whether a longer 18–28 °C transfer, over several weeks, similar to conditions existing for natural carp spawning during springtime, would result in a longer stimulation of muscle growth.

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