

Post-Hatching Development of Motor Innervation of Lateral Muscle in the Seabream *Sparus aurata*

Paolo Berardinelli, Giuseppe Radaelli⁽¹⁾, Anthea Rowlerson⁽²⁾, Pier Augusto Scapolo, Francesco Mascarello⁽¹⁾, and Alba Veggetti⁽³⁾

Dipartimento di Strutture, Funzioni e Patologie Animali e Biotecnologie, Università di Teramo, Piano D'Accio (TE), Italy, (1) Dipartimento di Scienze Zootecniche, Università di Padova, Legnaro, Italy, (2) Applied Clinical Anatomy, GKT School of Biomedical Sciences, Hodgkin Building, Guy's Campus, London, U.K., and (3) Dipartimento di Morfofisiologia Veterinaria e Produzioni Animali, Università di Bologna, Ozzano-Emilia, Italy

Abstract

In many fish, both production of new muscle fibres and neurogenesis continue into juvenile life. To test the hypothesis that new motoneurons are produced to supply the expanding muscle target we used the seabream (*Sparus aurata*), which shows a many-fold increase in the number of fibres in lateral muscle during posthatching juvenile development. A motor nerve branch innervating a segment of epaxial lateral white muscle was identified, and the type and number of its axons were measured in fish of several larval and post-larval ages. Contrary to expectation, total axon number was greatest in the larval fish (114.3 ± 22.6); unmyelinated axons were found only in the larval nerves, and the number of myelinated axons increased only modestly over the ages examined, from 58.5 ± 12.4 in larval fish to 77.5 ± 7.3 in post-larval juveniles. We conclude that in seabream the larval nerve still includes axons of motoneurons destined to die during the normal developmental phase of target-dependence in addition to those axons which will survive into juvenile life, and that the definitive number of motoneurons is already present in the larval fish before the main increase in muscle fibre number occurs.

Key words: axon, larval, motoneuron, neurogenesis, post-larval.

Basic Appl Myol 10 (6): 279-284, 2000

In many fish which grow to a large final size, production of new muscle fibres (muscle hyperplasia) continues for a prolonged period into adult life [14, 25, 29, 33, 34, 35]. Neurogenesis is also reported to be prolonged in lower vertebrates, and this has been observed in several areas of the CNS [5, 7, 11, 36]. It might therefore be predicted that, in association with the increase in target size (i.e. the number of muscle fibres) which occurs during growth, these fish could increase the number of motoneurons supplying the muscle as well as expand existing motoneuronal territories.

The development of muscle innervation has been most extensively studied in the zebrafish (*Brachydanio rerio*); motoneurons are formed early in development [21], and the number of motoneurons innervating muscle then stabilises soon after hatching [32, 37]. In the angelfish (*Pterophyllum scalare*) events are similar except for an excess production of motoneurons in the

early stages, but again the final number of axons is reached by 20 days after hatching [27]. However, zebrafish and angelfish are small fish, and show relatively little hyperplastic muscle growth post-hatching, so the size of the (muscle) target does not increase greatly after initial innervation is established. Observations made on the lateral (i.e. main trunk) muscle of fish which do grow to a large size have revealed: (i) a continuous increase in motor axon numbers throughout life in the stingray [16], (ii) a prolonged but gradually declining phase of motoneuron production which eventually ceased in adult life in the dogfish [20], (iii) a motoneuronal hyperplasia correlated with muscle growth in juvenile trout [1], and (iv) no significant change in motoneuron numbers in the eel despite several-fold increases in muscle fibre numbers [28]. Unfortunately, because these studies used different methods (axon vs motoneuron counts) as well as very different species (the dogfish

Motor innervation of the seabream muscle

and ray are elasmobranchs, the others teleosts), it is difficult to evaluate the significance of the discrepancies between the results obtained.

The main objective of the study described here was to test the hypothesis that a marked expansion in the peripheral target of motoneurons in a teleost fish would be accompanied by a concomitant increase in their number. We used the seabream (*Sparus aurata* L) because this teleost fish does grow to a large size, it is known to show a many-fold increase in the number of fibres in lateral muscle during postlarval development [26] and it is commercially farmed so that appropriate developmental stages raised under well-defined conditions are available. A nerve branch innervating epaxial lateral white muscle was selected for study at a position close to its exit from the spinal cord, where it contained only motor axons (see Discussion), and the number and diameter of its myelinated and unmyelinated axons were measured in fish of several larval and post-larval ages from 40 days post-hatching to 2 years.

Materials and Methods

Fish

Sparus aurata ranging in age from 40 days post-hatching to more than 2 years old were obtained from a commercial fish farm on the Adriatic coast of Italy, anaesthetised by immersion in MS222 (Sandoz) and killed by decapitation. Metamorphosis occurs at about 50 days post-hatching in this species, and the post-larval hyperplastic growth phase of lateral muscle starts shortly afterwards [26]. By 90 days all fry have reached this phase. The number and ages of fish used for axon counts are shown in Table 1, together with body weights for fish of these ages. Acetylcholinesterase staining [23] was carried out on paraformaldehyde-fixed muscle of fish aged 40, 60 and 100 days to check for any change in the distribution of innervation over the transition from larval to juvenile life, but revealed the distributed innervation typical of fish white muscle at all these ages.

Electronmicroscopy

Whole larvae and small fry, and small tissue blocks containing the appropriate area of spinal cord and epaxial muscle dissected out of larger fish immediately posterior to the anal vent, were fixed in 2.5% glutaraldehyde, 4% paraformaldehyde in 0.1M cacodylate buffer, pH 7.4 at 4°C for 3 hours. The samples were then rinsed overnight in cacodylate buffer, treated with 1% osmium tetroxide for 2 hours, and rinsed again in cacodylate buffer before being dehydrated and embedded in epon-araldite resin.

Blocks were sectioned in a plane giving transverse sections of the chosen nerve branch (DRv) shortly after its separation from the ventral root, as shown in figure 1a. Blocks of whole fish (larvae and small fry) were

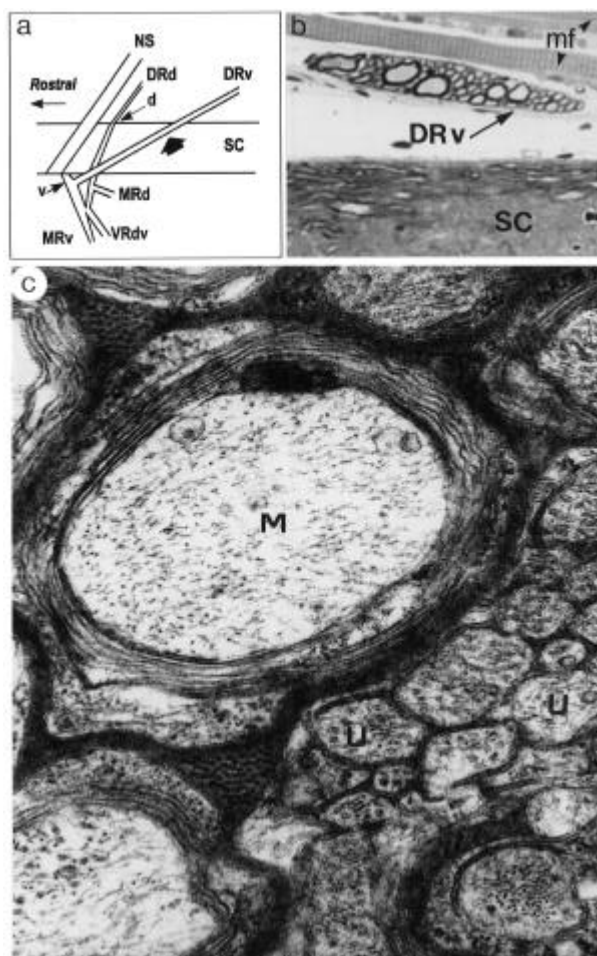


Figure 1. a) the arrangement of dorsal and ventral roots of the spinal nerves, and their main branches, as described by Fetcho [8]; the point at which the DRv nerve was sectioned is indicated by the bold arrow; b) the DRv nerve in transverse section at the position indicated in (a), lying between the membranous neural arch and deep epaxial muscle, as it appears in semi-thin sections stained with toluidine blue; c) myelinated (M) and unmyelinated (U) axons as seen with the electron microscope in a thin section of the DRv nerve in a 46 day larva. Other abbreviations (according to Fetcho, [8]): NS: neural spine; SC: spinal cord; v: ventral root; DRv: dorsal branch of ventral root (motor); MRv, VRdv: medial and ventral branches of the ventral ramus (mixed); d: dorsal root; DRd, MRd: dorsal and medial branches of the dorsal root (sensory); mf: muscle fibres. Magnifications: (b) 650 x; (c) 60,000 x.

initially trimmed in transverse section to the rostro-caudal axis until the anal vent was reached, and then tilted to the appropriate plane. Semi-thin (1 μ m) sections from all blocks were stained with toluidine blue to check that the level and plane of section were correct,

Motor innervation of the seabream muscle

Table 1. Axon numbers, types and diameters by fish age and weight¹.

	AGE OF FISH					
	Larval			Juvenile		
	<i>46d</i>	<i>56d</i>	<i>60d</i>	<i>100d</i>	<i>150d</i>	<i>700d</i>
body weight ²	~0.01g	~0.03g	~0.04g	~0.6g	~3g	120-400g
no. axons M ³ /U ⁴	52/30	52/73	44/64 ⁵	72/0	84/0	82/0
	48/31	71/56	42/66 ⁶	67/0	70/0	83/0
		64/90	65/43 ⁶	68/0		84/0
			70/50 ⁷	85/0		84/0
			77/55 ⁷	70/0		
				81/0		
mean diam. M ⁸	1.14±0.79	n	2.38±1.31	3.3±1.5	3.5±1.8	10.4±3.4
mean diam. U ⁹	0.26±0.08	n	n	-	-	-
mean no. M ¹⁰	58.5±12.4			77.5±7.3		
mean no. U ¹¹	55.8±18.6			0		

¹: includes data of Berardinelli et al. [4] and additional data for larval ages; ²: typical body weight for age; ³: M = myelinated, individual values for each fish; ⁴: U = unmyelinated, individual values for each fish; ⁵: body weight 0.051 g; ⁶: body weight 0.03 g; ⁷: body weight 0.02 g; ⁸: M = mean diameter (±s.d.) in µm for all myelinated axons at that age (except for 60 d, where value is derived from only 2 nerves containing a total of 142 axons); ⁹: U = mean diameter (±s.d.) in µm for unmyelinated axons at that age; ¹⁰: mean number (±s.d.) for all 'larval' fish from 46 - 60 days; ¹¹: mean number (±s.d.) for all postlarval fish from 100 days; n = not determined.

before cutting thin (70-90 nm) sections which were then contrasted with uranyl acetate and lead citrate.

Thin sections were viewed in a Zeiss EM900 electron microscope at magnifications ranging from 3000x to 50000x, and the nerve photographed on Kodak SO 163 film. The entire cross-section of the nerve was then reconstructed from the photographs, and all axons present were identified as myelinated or unmyelinated, and their diameter measured using a Digicad Plus digitising tablet linked to Videoplan software. If the axon was myelinated, the diameter measured included the myelin sheath. In the case of the older fish, once examination of thin sections in the EM had shown that no unmyelinated axons were present, diameter measurements were made with the same system from prints of serial semi-thin sections photographed in the light microscope (with a 100x objective). Direct comparison of myelinated axon diameters made on photographs of thin (EM) and semithin (LM) was made with two samples, and showed no significant difference in values.

Results

The appearance of a 100 day nerve *in situ* at light microscope level is illustrated in figure 1b. The electron-micrograph in figure 1c shows a portion of this nerve in a 46 day larva. This nerve contained both myelinated and unmyelinated axons; when present, unmyelinated axons usually occurred in 2 or 3 groups, not singly. Unmyelinated axons were found in all the subjects in the age range 46-60 days, but not in any older fish. The

numbers of axons of both types in fish at each age examined are shown in Table 1.

Examination of the epaxial muscle in all these subjects confirmed that the post-larval hyperplasia which results in the appearance of new fibres throughout the myotome had not started in the fish aged 60 days or less, but was underway in all fish aged 100 days or more. Axon numbers were therefore compared for these two groups of fish: post-larval (≥ 100 days) and 'larval' (≤ 60 days; strictly, some of these fish had completed metamorphosis, but the stage of muscle growth was still typical of the late larval stage). If only myelinated axons are taken into consideration, there was a modest but significant increase in their number in the older fish: mean values (± s.d.) were 58.5 ± 12.4 in larval fish and 77.5 ± 7.3 in the postlarval fish. Though small, this difference was significant as evaluated by student's t-test (P < 0.001, unpaired [30]). However, there was also a substantial, though quite variable, population of unmyelinated axons in the larval nerve (see Table 1), and if these are taken into account, the total number of axons (114.3 ± 22.6) in the nerve was significantly greater in the larval fish (P < 0.001, unpaired and corrected for unequal variances [30]).

Unmyelinated axons were all of very small diameter (< 1 µm). Measurement of myelinated axon diameters revealed a gradual increase with age, as summarised in Table 1. Mean diameter, minimum diameter and the largest diameter were all more than two-fold greater in the 60 day nerve with an 'adult' number (77) of myelinated axons than in the 46 day larvae. The smallest

Motor innervation of the seabream muscle

myelinated axon diameter was 0.53 μm , found in one of the 46 day larvae. It was also observed that the number of lamellae within myelin sheaths was least in the larval fish, but this was not quantified.

Discussion

Identity of axons

An important issue is the identification of the axons we counted in the DRv nerve as motor axons. The equivalent branch in other fish is considered to be the main motor nerve branch innervating epaxial muscle (teleosts: [3, 8]; elasmobranch: [20]) and several observations suggest that the nerve contained only motor axons at the point we examined in the seabream. Axon counts were made at the point shown in figure 1a, i.e. very shortly after the point where the DRv nerve branch separates from the ventral root shortly after its exit from the spinal cord, and before the point where the remaining ventral root axons are joined by dorsal root axons to form the (mixed) ventral and medial rami. The size distribution and type of axons in our oldest fish (i.e. all myelinated, and containing large diameter axons) was also typical of motor rami, rather than sensory ones (which contain small diameter myelinated and unmyelinated axons) [8]. Furthermore, because the DRv branch selected supplies only epaxial territory in a segment caudal to the abdomen, the presence of visceral afferents or a substantial autonomic component can also be excluded. Sakamoto et al. [27] have also shown that in ventral roots of the angelfish at anal vent level the number of axons is equal to the number of motoneurons.

Developmental changes in axon morphology

Our observations that the larval nerves contain both unmyelinated axons and axons with only thin myelin sheaths (figure 1c), and that even myelinated axons are very small in diameter (Table 1), indicate that the nerve is very immature at 46 days and that the process of myelination of axons is still at an early stage. In developing nervous systems, myelination starts soon after axon formation once a critical diameter has been exceeded, and is accompanied by a gradual increase in the number of membrane layers as the axon diameter increases further [6, 10, 17]. In mammals, myelination generally occurs at a smaller axon diameter in central axons (about 0.3 μm) than in peripheral axons (about 1 μm), but there is little comparable information about myelination in fish axons. The smallest diameter axon in the spinal motor nerve we examined was about 0.5 μm (and 26% of the axons in the 46d nerves had diameters less than 1 μm), which is significantly less than for mammalian peripheral axons, but slightly larger than the values of 0.2 μm [10] and 0.3 μm [12] observed in two cranial nerves of growing fish. Axon diameter subsequently increased with age (Table 1).

Numbers of axons

If only myelinated axons are taken into account, there was a modest increase in motor axon numbers in the postlarval fish in which muscle fibre hyperplasia had occurred. However, the size of the increase (about 30%) is far smaller than the increase in muscle fibre number (several-fold, [26]), suggesting that these changes were not causally linked. Given the immature state of the nerve in the larval fish, it is most likely that the unmyelinated population seen at that age contains axons which will contribute to the final myelinated population in postlarval fish, but which have not yet acquired a myelin sheath. In other words, the combined myelinated and unmyelinated population present in larval fish already included all of the 77 or so motor axons seen in this nerve in post-larval fish.

The population of unmyelinated axons in the larval nerves was very variable, however, and overall (mean number 55.8) exceeded the difference (19 axons) in myelinated axon numbers between larval and postlarval fish. How can this discrepancy be accounted for? Although the myelinated axons were easily identified and counted at all ages, the very small diameter and grouped nature of unmyelinated axons made them more difficult to identify. We cannot exclude the possibility that there was a small error in unmyelinated axon counts, but when the counts were made by two individuals on the same nerve there was quite good agreement, and we estimate that counting errors could account for at most a 10% difference. The use of the anal vent as a marker to identify the equivalent nerve and myotome in each case (see Methods) also ensured that errors due to variable position along the rostro-caudal axis were avoided.

Even in these commercially raised fish there can be large differences in body size and developmental status between fish of the same age. To reduce the effects of this variation we normally take only the larger (faster-growing) fish from any cohort, but an additional comparison of unmyelinated axon counts for some subjects of known body weight at the same age was also made to see if there was a relation between unmyelinated axon number and body size. However, no simple relation between unmyelinated axon number and either age or size was evident within the larval fish population (see Table 1).

Most probably, the variable number of unmyelinated axons in DRv nerve is the result of two overlapping but not synchronous processes which both affect unmyelinated axon numbers: normal developmental motoneuronal death [22] resulting in loss of axons, and gradual myelination of axons of motoneurons which will survive. Work on other vertebrates suggests that there is an initial over-production of motoneurons by a factor of 2 or more [2, 18, 22]. Although most of the developing motoneurons do make functional contact with their target muscle [2, 6, 19], many will fail to make sufficient peripheral

Motor innervation of the seabream muscle

contacts to survive the subsequent period of target dependence and will therefore die [15, 24, 31]. Developmental cell death has also been found in the electromotor neuron pool of the fish *Torpedo marmorata*, although it accounted for a smaller proportion of cells [11]. Finally, in the angelfish investigated by Sakamoto et al. [27], there was a roughly 40% fall in motor axons number in the immediate post-hatching period. We therefore suggest that the unmyelinated axon population in the larval DRv nerve includes axons of motoneurons which would not have survived the phase of target-dependence in addition to axons which would have survived but had not yet begun myelination, a situation similar to that described for ventral roots in the embryonic chicken [6].

Given the general context of known persistence of neurogenesis in fish, and the studies which have described motoneuronal hyperplasia linked to muscle growth in fish [1, 9, 16, 20] and in another lower vertebrate (axolotl: [13]), we were surprised to obtain results suggesting that the definitive number of motoneurons is already present before the main increase in muscle fibre number occurs. However, an estimate of the corresponding ratio of motoneurons to muscle fibres, indicates that the number of axons is within the expected range for the adult number of muscle fibres innervated.

Although we do not know the exact area within the myotome supplied by the DRv nerve, we can assume that it supplies a substantial part of the epaxial muscle (as described for cat-fish, [3, 8]), and we do know how epaxial white muscle fibre number increases during development [26]. Supposing that the DRv nerve supplies, say, the apical 50% of epaxial muscle in its myotome, this is equivalent to about 420 fibres at 60 days and 3500 fibres by 150 days, giving ratios of motor axons : muscle fibres of 1:5.4 and 1:45.2, respectively. As muscle fibre hyperplasia continues beyond 150 days, the final ratio could reasonably reach at least double that value i.e. about 1:100. This is well within the range for many mammalian motor units, and reasonably close to the ratio in zebrafish, which is about 1:50 (assuming that half of the 71 motor axons per ventral root [37] supply one epaxial quadrant of the myotome which contains about 1700 fibres [32]).

In conclusion, our results suggest that in the seabream *Sparus aurata*, the definitive number of motoneurons is already present and in contact with the lateral muscle in the larval fish, well before the main increase in muscle fibre number occurs. This is contrary to the results of Alfei et al. [1], but both supports and extends the conclusions reached by Smit et al. [28], who found no change in motoneuronal numbers during postlarval growth in the eel (another teleost), but were not able to study larval stages. The possibility remains that motoneuronal hyperplasia may be genuinely prolonged in large elasmobranchs [16, 20], and it would be interest-

ing to see if future studies confirm a difference between elasmobranchs and teleosts in this respect.

Acknowledgements

We thank the following organisations for financial support: BBSRC (AMR: no. AG/35/501), MURST-60% (PAS) & MURST-40% (AV)

Address correspondence to:

Prof. P.A. Scapolo, Dipartimento di Strutture, Funzioni e Patologie Animali e Biotecnologie, Università degli Studi di Teramo, 64020 Piano D'Accio (TE), Italy, tel. 00390861266850, fax 00390861266860, Email scapolo@ianve.vet.unite.it.

References

- [1]Alfei L, Bertoncetto G, Gelosi E, Parvopassu F: A morphometric analysis of the spinal motor pool in relation to its target muscle during growth in the trout, *Salmo gairdneri* Richardson. *J Fish Biol* 1989; 34: 65-78.
- [2]Ashwell KW, Watson CRR: The development of facial motoneurons in the mouse - neuronal death and the innervation of the facial muscles. *J Embryol exp Morph* 1983; 77: 117-144.
- [3]Barets A: Contribution a l'étude des systèmes moteurs 'lent' et 'rapide' du muscle latéral des Téléostéens. *Arch Anat Microsc Morph Exp* 1961; 50: 92-187.
- [4]Berardinelli P, Martelli A, Radaelli G, Rowleson A, Scapolo P-A: Number and diameter of axons innervating lateral muscle of the sea bream (*Sparus aurata*) during development. *J Physiol* 1997; 504: 14-15P.
- [5]Birse SC, Leonard RB, Coggeshall RE: Neuronal increase in various areas of the nervous system of the guppy, *Lebistes*. *J Comp Neurol* 1980; 194: 291-301.
- [6]Chu-Wang IW, Oppenheim RW: Cell death of motoneurons in the chick embryo spinal cord. II. A quantitative and qualitative analysis of degeneration in the ventral root, including evidence for axon outgrowth and limb innervation prior to cell death. *J Comp Neurol* 1978; 177: 59-86.
- [7]Easter SS: Postnatal neurogenesis and changing connections. *Trends Neurosci* 1983; 6: 53-56.
- [8]Fetcho JR: The organization of the motoneurons innervating the axial musculature of vertebrates. I. Goldfish (*Carassius auratus*) and mudpuppies (*Necturus maculosus*). *J Comp Neurol* 1986; 249: 521-550.
- [9]Fine ML: Embryonic, larval and adult development of the sonic neuromuscular system in the oyster toadfish. *Brain Behav Evol* 1989; 34: 13-24
- [10]Finger TE, Drake SK, Kotrschall K, Womble M, Dockstader KC: Postlarval growth of the peripheral gustatory system in the channel catfish, *Ictalurus punctatus*. *J Comp Neurol* 1991; 314: 55-66.
- [11]Fox GQ, Richardson GP: The developmental morphology of *Torpedo marmorata*: electric lobe elec-

Motor innervation of the seabream muscle

- tromotoneuron proliferation and cell death. *J Comp Neurol* 1982; 207: 183-190.
- [12]Hildebrand C, Wiberg J, Holje L: Trigeminal alveolar nerve of the lower jaw in the cichlid *Tilapia mariae*: evidence for continual axon generation and presence of exceptionally small myelinated axons. *J Comp Neurol* 1988; 272: 309-316.
- [13]Holder N, Clarke JDW, Stephens N, Wilson S, Orsi C, Bloomer T, Tonge DA: Continuous growth of the motor system in the axolotl. *J Comp Neurol* 1991; 303: 534-550.
- [14]Koumans JTM, Akster HA, Booms RGH, Osse JWM: Influence of fish size on proliferation and differentiation of cultured myosatellite cells of white axial muscle of carp *Cyprinus carpio* L.). *Differentiation* 1993; 53: 1-6.
- [15]Landmesser LT, Dahm L, Tang J, Rutishauser U: Polysialic acid as a regulator of intramuscular nerve branching during embryonic development. *Neuron* 1990; 4: 655-667.
- [16]Leonard RB, Coggeshall RE, Willis WD: A documentation of an age related increase in neuronal and axonal numbers in the stingray, *Dasyatis sabina*, Lesueur. *J Comp Neurol* 1978; 179: 13-22.
- [17]Matthews MA: An electron microscopic study of the relationship between axon diameter and the initiation of myelin production in the peripheral nervous system. *Anat Rec* 1968; 161: 337-352.
- [18]McLennan IS: Size of motoneuron pool may be related to numbers of myotubes in developing muscle. *Dev Biol* 1982; 92: 263-265.
- [19]McLennan IS: Quantitative relationships between motoneuron and muscle development in *Xenopus laevis*: implications for motoneuron cell death and motor unit formation. *J Comp Neurol* 1988; 271: 19-29.
- [20]Mos W, Williamson R: A quantitative analysis of the spinal motor pool and its target muscle during growth in the dogfish, *Scyliorhinus canicula*. *J Comp Neurol* 1986; 248: 431-440.
- [21]Myers PZ, Eisen JS, Westerfield M: Development and axonal outgrowth of identified motoneurons in the zebrafish. *J Neurosci* 1986; 6: 2278-2289.
- [22]Oppenheim RW: Cell death during development of the nervous system. *Annu Rev Neurosci* 1991; 14: 453-501.
- [23]Pécot-Dechavassine M: Étude biochimique, pharmacologique et histochimique des cholinesterase des muscle striés chez le poissons, les bactériens et les mammifères. *Arch Anat Microscop Morphol Exp* 1961; 50S: 341-438.
- [24]Pittman R, Oppenheim RW: Cell death of motoneurons in the chick embryo spinal cord. IV. Evidence that a functional neuromuscular interaction is involved in the regulation of naturally occurring cell death and the stabilization of the synapses. *J Comp Neurol* 1979; 187: 425-446.
- [25]Romanello MG, Scapolo PA, Luprano S, Mascarello F: Post-larval growth in the lateral white muscle of the eel, *Anguilla anguilla*. *J Fish Biol* 1987; 30: 161-172.
- [26]Rowlerson A, Mascarello F, Radaelli G, Veggetti A: Differentiation and growth of muscle in the fish *Sparus aurata* (L): II. Hyperplastic and hypertrophic growth of lateral muscle from hatching to adult. *J Muscle Res Cell Motil* 1995; 16: 223-236.
- [27]Sakamoto H, Yoshida M, Sakamoto T, Uematsu K: Development of the myotomal neuromuscular system in embryonic and larval angelfish, *Pterophyllum scalare*. *Zool Sci* 1999; 16: 775-784.
- [28]Smit WA, Roberts BL, Velzing EH: Changes in size and number of spinal motoneurons in relation to growth of the musculature in the eel, *Anguilla*. *Dev Brain Res* 1991; 58: 73-80.
- [29]Stickland NC: Growth and development of muscle fibres in the rainbow trout (*Salmo gairdneri*). *J Anat* 1983; 137: 323-333.
- [30]Swinscow TDV: Statistics at square one. 9th edition, BMJ publishing Group, London, 1996.
- [31]Tanaka H, Landmesser LT: Cell death of lumbosacral neurons in chick, quail and chick-quail chicken embryos: a test of the quantitative matching hypothesis of neuronal cell death. *J Neurosci* 1986; 6: 2889-2899.
- [32]van Raamsdonk W, Mos MJ, van der Laarse WJ, Fehres R: The development of the spinal motor column in relation to the myotomal muscle fibers in the zebrafish (*Brachydanio rerio*). *Anat Embryol* 1983; 167: 125-139.
- [33]Veggetti A, Mascarello F, Scapolo PA, Rowlerson A: Hyperplastic and hypertrophic growth of lateral muscle in *Dicentrarchus labrax* (L.). An ultrastructural and morphometric study. *Anat Embryol* 1990; 182: 1-10.
- [34]Weatherley AH, Gill HS, Rogers SC: The relationship between mosaic muscle fibres and size in rainbow trout (*Salmo gairdneri*). *J Fish Biol* 1980; 17: 603-610.
- [35]Weatherley AH, Gill HS, Lobo AF: Recruitment and maximal diameter of axial muscle fibres in teleosts and their relationship to somatic growth and ultimate size. *J Fish Biol* 1988; 33: 851-859.
- [36]Weis JS: Analysis of the development of the nervous system of the zebrafish, *Brachydanio rerio*. *J Embryol exp Morphol* 1968; 19: 109-119.
- [37]Westerfield M, McMurray JV, Eisen JS: Identified motoneurons and their innervation of axial muscles in the zebrafish. *J Neurosci* 1986; 6: 2267-2277.