

# Fiber-Type Distribution of the Perivertebral Musculature in *Ambystoma*

Nadja Schilling<sup>1\*</sup> and Stephen M. Deban<sup>2</sup>

<sup>1</sup>*Institute of Systematic Zoology and Evolutionary Biology, Friedrich-Schiller-University, Erbertstr. 1, 07743 Jena, Germany*

<sup>2</sup>*Department of Integrative Biology, University of South Florida, Tampa, Florida 33620*

**ABSTRACT** Many salamanders locomote in aquatic and terrestrial environments. During swimming, body propulsion is solely produced by the axial musculature generating lateral undulations of the trunk and tail. During terrestrial locomotion, the trunk is oscillated laterally in a standing wave, and body propulsion is achieved by concerted trunk and limb muscle action. The goal of this study was to increase our knowledge of the functional morphology of the tetrapod trunk. We investigated the muscle-fiber-type distribution and the anatomical cross-sectional area of all perivertebral muscles in *Ambystoma tigrinum* and *A. maculatum*. Muscle-fiber-type composition was determined in serial cross-sections based on m-ATPase activity. Five different body segments were investigated to test for cranio-caudal changes along the trunk. The overall fiber-type distribution was very similar between the species, but *A. tigrinum* had relatively larger muscles than *A. maculatum*, which may be related to its digging behavior. None of the perivertebral muscles possessed a homogeneous fiber-type composition. The *M. interspinalis* showed a distinct layered organization and may function to ensure the integrity of the spine (local stabilization). The *M. dorsalis trunci* exhibited the plesiomorphic pattern for notochordates in having a distinct superficial layer of red and intermediate fibers, which covered the central white fibers; therefore, it is suggested to function as a mobilizer and a stabilizer of the trunk, but, may also be involved in modulating body stiffness. Similarly, the *M. subvertebralis* showed clear regionalizations, implying functional subunits that can stabilize and mobilize the trunk as well as modulate of body stiffness. Cranio-caudally, neither the fiber-type composition nor the a-csa changed dramatically, possibly reflecting the need to perform well in both aquatic and terrestrial habitats. *J. Morphol.* 271:200–214, 2010. © 2009 Wiley-Liss, Inc.

**KEY WORDS:** enzyme histochemistry; epaxial; hypaxial; urodele; amphibian

## INTRODUCTION

Salamanders are a key taxon in studies of vertebrate evolution by virtue of their generalized tetrapod body form, phylogenetic position, and the amphibious mode of life of many species. In studies of locomotor morphology and function, they

have served as models of ancestral tetrapods which move through both aquatic and terrestrial habitats and must meet the physical demands of these two environments. During swimming, thrust is solely generated by the axial musculature that produces lateral undulations of the trunk and tail, while the limbs are pressed against the body (Gray, 1944). These lateral undulations form traveling waves that display a caudad increasing amplitude of lateral excursion (Frolich and Biewener, 1992; D'Aout and Aerts, 1997). During terrestrial locomotion, salamanders bend the trunk from side to side in standing waves, while the limbs serve as anchors but also contribute to stride length (Barclay, 1946; Evans, 1946; Roos, 1964). Body propulsion is thus achieved by concerted trunk and limb muscle action (Gray, 1944; Barclay, 1946; Evans, 1946). At the same time, trunk bending results in part by the action of the extrinsic limb muscles (Barclay, 1946; Gray, 1968). Corresponding to these two modes of trunk bending during aquatic and terrestrial locomotion, the musculature is activated sequentially or simultaneously along the trunk (Frolich and Biewener, 1992; Delvolve et al., 1997; D'Aout and Aerts, 1997).

The axial muscles responsible for trunk movements have been investigated from several perspectives including anatomy, physiology, and biomechanics (e.g., Maurer, 1892; Nishi, 1916; Aufenberg, 1959; Carrier, 1993; D'Aout et al., 1996; Simons and Brainerd, 1999; Bennett et al., 2001;

---

Contract grant sponsor: Center of Interdisciplinary Prevention of Diseases related to Professional Activities funded by the Friedrich-Schiller-University Jena and the Berufsgenossenschaft Nahrungsmittel und Gaststätten Erfurt (Germany).

\*Correspondence to: Nadja Schilling, Institut für Spezielle Zoologie und Evolutionsbiologie mit Phyletischem Museum, Friedrich-Schiller-Universität Jena, Erbertstr. 1, 07743 Jena, Germany.  
E-mail: nadja.schilling@uni-jena.de

Received 1 May 2009; Revised 14 July 2009;  
Accepted 17 July 2009

Published online 25 August 2009 in  
Wiley InterScience (www.interscience.wiley.com)  
DOI: 10.1002/jmor.10791

Azizi and Brainerd, 2007). Nonetheless, little is known about the histochemical profile of salamander trunk muscles reflected by the proportion and distribution of the different fiber types. Because muscle fiber types differ, for example, in their innervation pattern, action potential propagation, mitochondrial density, and activity of enzymes, the fiber-type composition will endow a muscle with particular contractile properties. Conversely, a muscle's function can be deduced from its composition and distribution of fiber types when integrated with other anatomical and physiological details (Burke, 1981). Muscle-fiber-type composition in salamander trunk muscles has only been examined in the epaxial M. dorsalis trunci, where its striking resemblance to the axial myomeres of other vertebrates was pointed out (Totland, 1976a; Flood et al., 1977). Other vertebral muscles have not been investigated. In the previous studies only one trunk segment was investigated and potential cranio-caudal changes in the histochemical profile, possibly reflecting different roles of body segments, were not examined.

Traditionally, three types of muscle fibers are distinguished in amphibian muscles and are referred to as: red, medium, and white; slow, intermediate, and fast; tonic, intermediate, and phasic; or I, IIa and IIb, respectively (Ogata and Mori, 1964; Asmussen and Kiessling, 1974; Totland, 1976a,b; Lännergren, 1979; Ashley-Ross and Barker, 2002). Depending on the technique and the species, four or five fiber types, that is, up to two varieties of slow and three varieties of fast fibers, were identified in anuran limb muscles (Smith and Ovalle, 1973; Rowleron and Spurway, 1988; Lutz et al., 1998), which, however, corresponded to only three types of motor units: slow (tonic), intermediate (twitch), and fast (twitch, phasic; Sherkov, 1970; Asmussen and Kiessling, 1974; Tonge et al., 1985). In urodelan muscles, only three different fiber types—red, intermediate, and white—were identified in previous studies (Totland, 1976b; Ashley-Ross and Barker, 2002) and these types will also be distinguished in this study to allow comparison to previous results in salamanders as well as in other vertebrate taxa. Red tonic fibers contract slowly, do not propagate action potentials, and are fatigue resistant, while white twitch fibers contract and fatigue quickly. Intermediate twitch fibers are intermediate in their properties. Accordingly, red and intermediate fibers were suggested to fulfill a tonic function in maintaining the body posture while white fibers are involved in movements (Sherkov, 1970). Accumulations of these fiber types in certain regions of the muscle provide evidence of functional subdivisions that are linked to different functions during an animal's behavior.

The goal of this study is to assemble a more complete picture of the histochemical profile of the

axial muscles in salamanders by investigating the muscle-fiber-type composition and distribution of all perivertebral muscles at several vertebral levels along the trunk in two closely related *Ambystoma* species (*A. tigrinum* and *A. maculatum*). These taxa were chosen because ambystomatids resemble the hypothesized ancestral condition of urodeles regarding their amphibious mode of life, locomotor behavior, and structure and development of their axial skeleton (Worthington and Wake, 1972). By comparing our results from this pivotal tetrapod taxon with data from other vertebrates, we will be able to test phylogenetic hypotheses regarding the evolution of the axial musculature in vertebrates.

## MATERIALS AND METHODS

### Animals

Two adult tiger salamanders (Ambystomatidae: *Ambystoma tigrinum* GREEN, 1825; female: 17.0 g, SVL (snout-vent-length): 88 mm; male: 14.8 g, SVL: 85 mm) and two adult spotted salamanders (*A. maculatum* SHAW, 1802; female: 19.9 g, SVL: 95 mm; male: 17.3 g, SVL: 93 mm) were used in this study. Additionally, anatomical dissections of two more individuals per species were undertaken to comprehend the anatomy of the muscles and identify the respective muscles in the histological sections. Diverse anatomical descriptions were consulted (e.g., Maurer, 1892; Maurer, 1911; Nishi, 1916; Francis, 1934; Auffenberg, 1959; Starck, 1978). All animals were purchased from licensed animal dealers and all procedures were in accordance to the guidelines of animal welfare of the state of Thuringia, Germany.

Serial cross-sections through the complete perivertebral musculature including the vertebral column were prepared, and thus the topographical relationships within and among the muscles were maintained. Animals were killed by immersion in water containing a lethal dose of the anesthetic Acetonchloroform (1,1,1 Trichlor-2-methyl-2-propanol-Hemihydrat; Fluka, Germany). Immediately after death, head, fore-, and hindlimbs were removed and the animals were eviscerated. The tails were transected caudal to the vent and used for adjusting the enzyme-histochemical protocol. To avoid damage of the axial muscles due to the tight attachment of the skin to the myosepta and of the superficial muscle fibers to the skin via the fascia dorsi (Nishi, 1916), the skin remained on the specimens. Abdominal wall muscles were cut off ventral to the sulcus lateralis. Therefore, laterally in the histological cross-sections, the remnants of the abdominal wall muscles and the M. rectus lateralis are visible (e.g., Fig. 1a). The specimens were quick-frozen in Isopentane (Fluka, Germany) cooled by liquid nitrogen and stored in liquid nitrogen until further processing.

### Histology

Histological cross-sectional series of the trunks were prepared using a cryostat microtome (SLEE MTE, D knife, 12  $\mu$ m). Three muscle fiber types, that is, red (slow, tonic), intermediate, and white (both fast, twitch) (Totland, 1976a,b), were identified according to the activity of their myofibrillar ATPase (System C by Snow et al., 1982). An acid milieu during preincubation inhibits the activity of the acid-labile mATPase (e.g., of the white fibers) but allows the acid-stabile mATPase to react (e.g., of red fibers), while the acid-labile and alkaline-stabile mATPase is active after preincubation in an alkaline milieu. As in fish (Johnston et al., 1974), the intermediate fibers were the most resistant to inactivation. The result is that red and intermediate muscle fibers were stained dark after acid

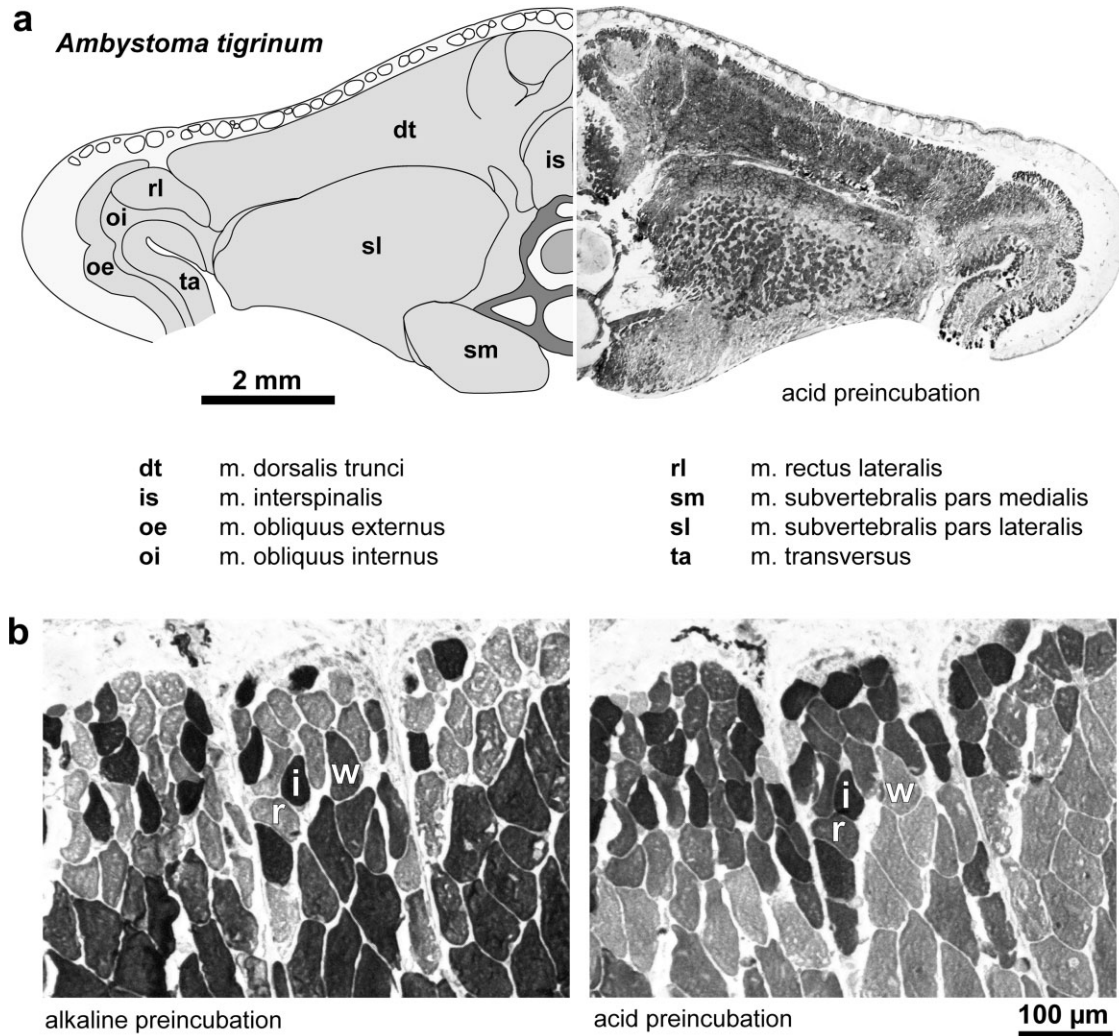


Fig. 1. (a) Histological cross-section after acid preincubation (right) and schematic drawing (left) illustrating the topography of the perivertebral musculature in example of *Ambystoma tigrinum* at the 4th external body segment. (b) Result of the enzyme-histochemical reaction after alkaline and acid preincubation. Note the complementary results of the staining: white (w) and intermediate (i) fibers are stained after alkaline preincubation, while red (r) and intermediate fibers are stained after acid preincubation. Comparison of successive sections allows identification of the fibers types.

preincubation due to the brown sublimate of the enzyme-histochemical reaction, while white muscle fibers were not stained. After alkaline preincubation, white and intermediate muscle fibers were stained whereas red muscle fibers were not stained (Fig. 1b). By comparing respective fibers in successive serial cross-sections from acid and alkaline preincubation, the different muscle-fiber types were identified. Several test series with different pH-values and durations for the preincubation revealed the best results at pH 10.4 for alkaline (10–15 min) and at pH 4.6 for acid preincubation (3 min) in both species.

### Fiber-Type Composition

The fiber-type distribution was investigated in the histological cross-sections using a Stemi SV 11 or an Axiolab microscope (Zeiss, Germany). Images of the complete histological cross-sections at selected cranio-caudal levels and of selected muscle areas to illustrate the typical pattern for that particular muscle were taken using digital cameras mounted to the microscopes (both ColorView, Soft Imaging System GmbH, Münster,

Germany:  $1,280 \times 1,024$  pixels and  $2,576 \times 1,932$  pixels, respectively). *A. tigrinum* has 14–15 and *A. maculatum* 14–16 trunk vertebrae, that is, vertebrae between the cervical and the sacral vertebrae (Worthington and Wake, 1972; Frolich and Biewener, 1992; Boisvert, 2009). The individuals investigated in this study possessed 15 trunk vertebrae, of which number 3–14 corresponded to the 12 external trunk segments visible, between their respective costal grooves.

The overall fiber-type distribution was investigated at five cranio-caudal levels covering the complete trunk between the limb girdles for both species. The cranio-caudal levels corresponded to external body segments 2, 4, 6, 8, and 10 (i.e., segments between costal grooves 2 and 3, 4 and 5, 6 and 7, 8 and 9, as well as 10 and 11; starting to count with one in the axillary groove). The quantitative analysis of the fiber-type composition focused on two body segments, that is, the 4th and 8th external body segments, to investigate whether the composition of fiber types changed along the trunk (see Fig. 2).

The fiber-type composition of the Mm. interspinalis, dorsalis trunci, subvertebralis pars lateralis et pars medialis was determined in an example of *A. tigrinum* because both species

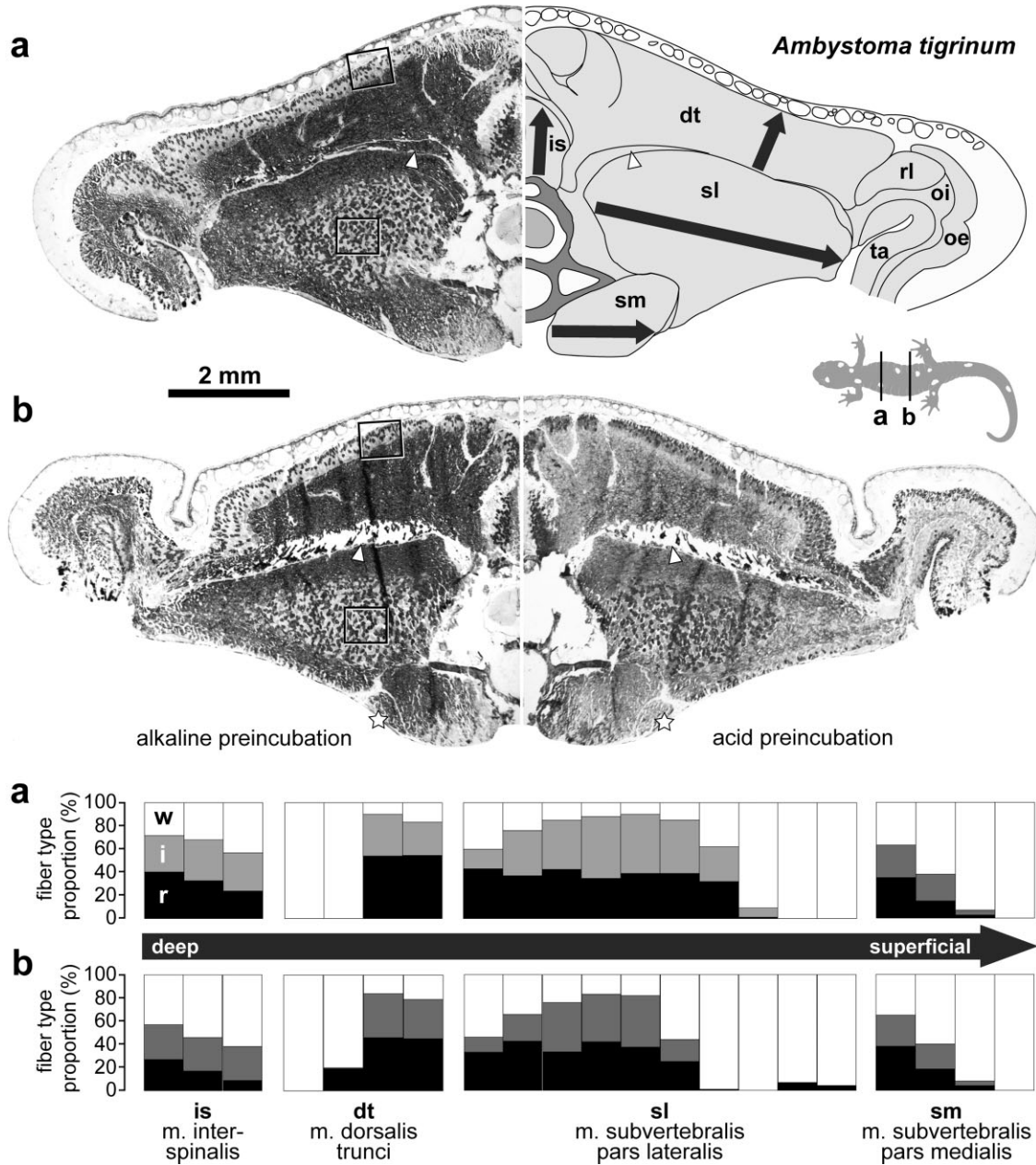


Fig. 2. Histological cross-sections and proportion (in %) of the white (w), intermediate (i), and red (r) muscle fibers along a region of interest in the respective perivertebral muscles of *Ambystoma tigrinum* at the 4th (a) and the 8th (b) body segments depicted as bar charts (note that the left sections are mirrored to complete the cross-section). Analyzed muscle areas extending from deep to superficial are indicated by thick gray arrows in this example of the 4th external trunk segment (muscle names abbreviated as in Fig. 1). The white arrowheads indicate the portion of the M. dorsalis trunci referred to as m. intertransversarius. The white stars at the bottom indicate the pars obliqua interna of the M. subvertebralis. The boxes on the left histological sections indicate the muscle areas analyzed for Figure 3.

appeared similar in their overall fiber-type composition (compare Figs. 4 and 5). Both individuals of *A. tigrinum* were similar in their fiber-type distribution (compare Figs. 2 and 5); therefore, only one was selected for this more detailed analysis (based on the overall quality of the staining in all cross-sections). Thus, the fiber-type percentages given in the results are from the female *A. tigrinum*. For this analysis, adjacent and corresponding images of a region ranging from the deep to the superficial were taken in the two consecutive histological cross-sections (i.e., one after alkaline and one after acid

preincubation) using the image analysis software analySIS (Olympus Soft Imaging Solutions GmbH, Münster, Germany). Each image contained 50–150 fibers. All fibers of the same type were identified by comparing the two respective images of the consecutive cross-sections and each type was counted. Double counting was prevented by semi-automatic marking of the fibers. The proportion of a certain fiber type was calculated as the percentage of all fibers present in one image (Figs. 2, 3). Similarly, the fiber-type composition was determined in two selected muscle areas, one superficial in the M. dorsalis trunci

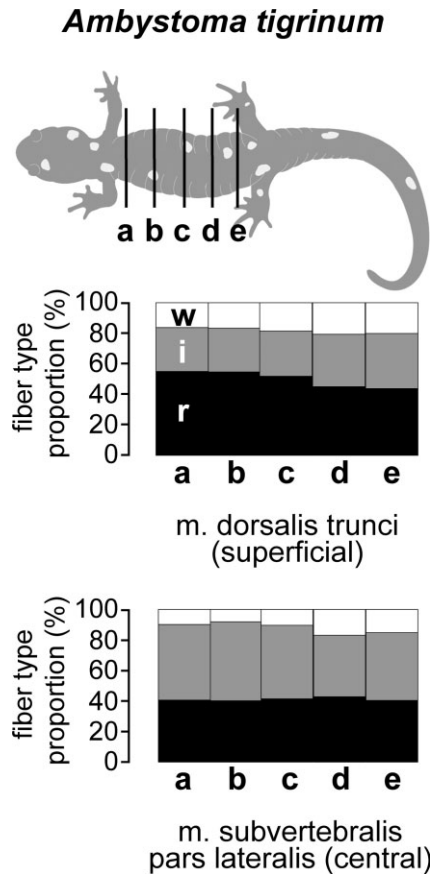


Fig. 3. Bar charts depict the proportion (in %) of the white (w), intermediate (i), and red (r) muscle fibers along the cranio-caudal axis in selected areas of the M. dorsalis trunci and the M. subvertebralis pars lateralis (for muscle areas see frames in Fig. 2). The respective muscle areas were analyzed at 5 different cranio-caudal levels referring to the **a**: 2nd, **b**: 4th, **c**: 6th, **d**: 8th, and **e**: 10th external segments of the animal. Note the minimal changes in the fiber-type composition in cranio-caudal direction in the M. dorsalis trunci and M. subvertebralis pars lateralis.

and one central in the M. subvertebralis pars lateralis (see frames in Fig. 2), at the five selected cranio-caudal levels to test if the muscle-fiber-type proportion changed along the body axis (see Fig. 3).

### Anatomical Cross-Sectional Area

Anatomical cross-sectional area (a-csa) was measured using ImageJ 1.41 (Wayne Rasband, NIH). Areas of the Mm. interspinalis, dorsalis trunci, subvertebralis pars lateralis et pars medialis were measured at the five different cranio-caudal levels illustrated in Figures 4 and 5 (male of *A. tigrinum*; heavier female of *A. maculatum*). All muscles in both consecutive histological cross-sections for the particular cranio-caudal level were measured (one after alkaline and one after acid preincubation). The mean of the areas determined per muscle in these two sections was calculated and expressed as relative a-csa ( $\text{mm}^2/\text{g}$  or  $\text{mm}^2/\text{mm}$ ), that is, measured area corrected by body mass or SVL, and as percentage of the total area of all perivertebral muscles (Table 1). Also calculated were the proportions of the epaxial muscles (Mm. interspinalis et dorsalis trunci) and the hypaxial muscles (M. subvertebralis partes medialis et lateralis) relative to the whole a-csa of the perivertebral musculature. Measuring the a-csa for all muscles five times independently in

the same histological cross-section revealed an average error of measurement of 3.8% for all muscles (M. interspinalis: 7.9%, M. dorsalis trunci: 2.0%, M. subvertebralis pars lateralis: 2.3%, M. subvertebralis pars medialis: 2.9%).

## RESULTS

### Cross-Sectional Fiber-Type Distribution

*Ambystoma maculatum* and *A. tigrinum* showed striking similarities in their distribution of muscle-fiber types (Figs. 4 and 5). All perivertebral muscles exhibited clear regionalizations (i.e., accumulations of one or two fiber types in a particular muscle area). Red and intermediate fibers were generally superficial in the epaxial muscles but deep or near the vertebrae in the hypaxial muscles. No major changes along the body axis of the muscle fiber type composition or distribution were found in either species (Figs. 4 and 5). The general distribution within a given muscle was maintained throughout the trunk and neither the location nor the extent of a particular regionalization changed clearly in the cranio-caudal direction (except the M. subvertebralis in *A. tigrinum*). Beside some minor changes in the shape of the muscles, each body segment appeared like a repetition of the previous body segment.

The quantitative comparison among the five different cranio-caudal levels in *A. tigrinum* confirms this observation for the central region of the M. subvertebralis pars lateralis and shows only minor changes for the superficial region of the M. dorsalis trunci (see Fig. 3). Although the proportion of the red fibers in the lateral subvertebral muscle varied little among the different body segments (40 to 43%), the proportion of the white and intermediate muscle fibers ranged from 8 to 17% and 40 to 52%, respectively. But again, no differences were observed in the percentage of a given fiber type at different cranio-caudal levels. In the superficial area of the M. dorsalis trunci, a slight increase of the proportion of the white muscle fibers from 16 to 20% and a somewhat greater increase in the proportion of the intermediate fibers from 29 to 36% in cranio-caudal direction were correlated with a decrease in red fibers (from 55% to 44%). The shift in the composition of the superficial dorsalis trunci muscle was thus mainly due to an increase of intermediate and a decrease of red muscle fibers. These changes were related to varying thicknesses of the superficial layer (i.e., depending on how close the investigated area was to a myoseptum as Fig. 5 illustrates) rather than to the segmental level along the trunk.

### M. interspinalis

The M. interspinalis is situated dorsal and lateral to the vertebrae (Nishi, 1916; Francis, 1934). The short, monosegmental fiber bundles arise from

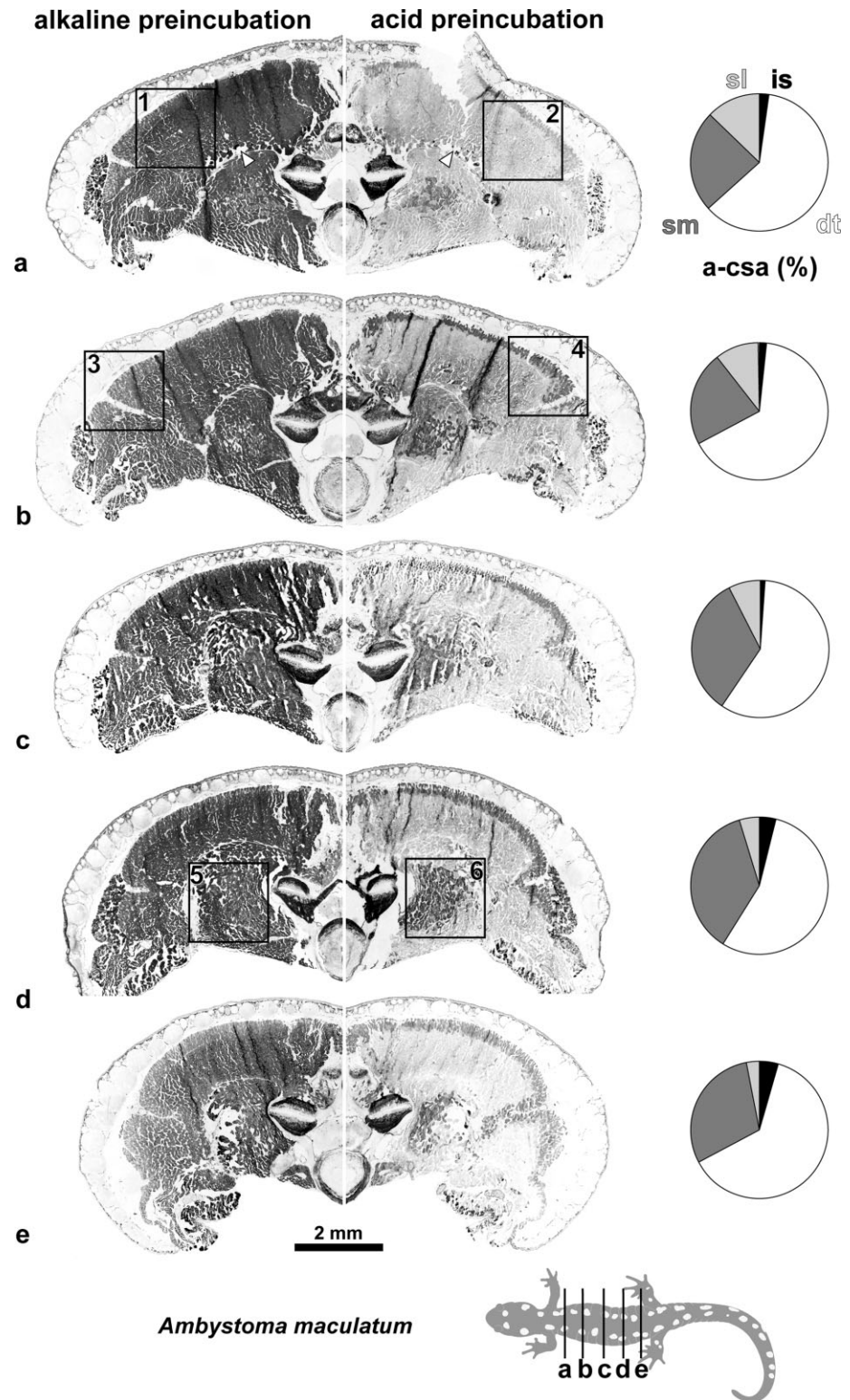


Fig. 4. Histological cross-sections of *Ambystoma maculatum* at five different cranio-caudal levels (a: 2nd, b: 4th, c: 6th, d: 8th, and e: 10th external segment) after alkaline (left, mirrored) and acid preincubation (right). Note the homogeneity of the fiber-type distribution within a given muscle along the trunk. The numbered frames refer to the images in Figure 6. The white arrowheads indicate the portion of the M. dorsalis trunci referred to as M. intertransversarius. Pie charts to the right depict the anatomical cross-sectional areas (a-csa) at the respective five cranio-caudal levels a to e of the M. interspinalis (is), M. dorsalis trunci (dt), M. subvertebralis pars lateralis (sl), and M. subvertebralis pars medialis (sm) as percentage of the area of all perivertebral muscles.

the postero-dorsal edge of the postzygapophysis of one vertebra and insert along the dorsal surfaces of the neural arch of the next caudad vertebra, thus filling the concavity between the zygapophyses and the neural spines (see Fig. 2). Their fibers

are also referred to as intervertebral fibers IV-1 and IV-2 (Auffenberg, 1959).

The interspinalis muscles showed a clear regionalization in the medio-lateral direction in both species (e.g., Figs. 2, 4d, 5c, and 6). Medially, next

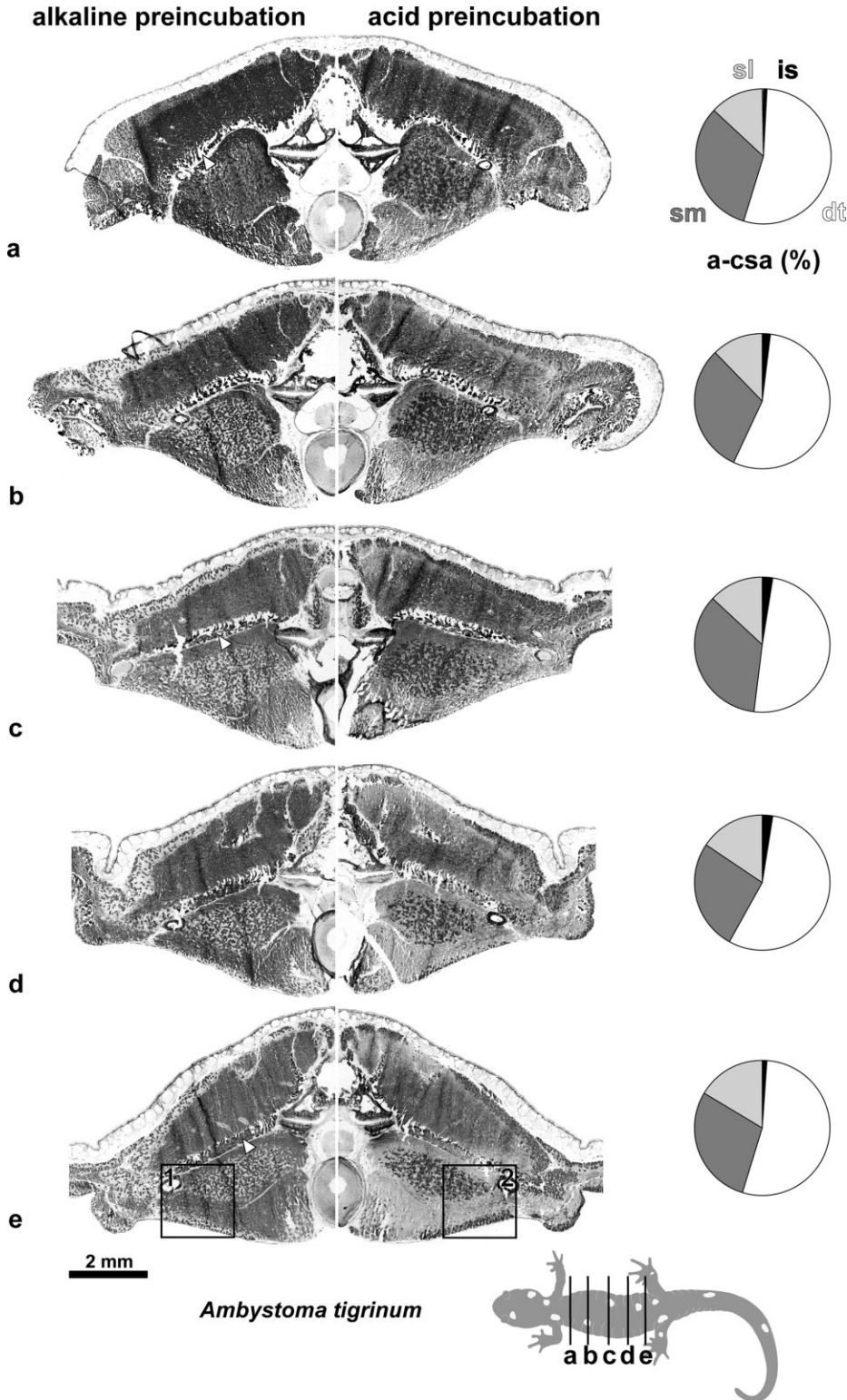


Fig. 5. Histological cross-sections of *Ambystoma tigrinum* at five different cranio-caudal levels (a: 2nd, b: 4th, c: 6th, d: 8th, and e: 10th external segment) after alkaline preincubation (left, mirrored) and acid preincubation (right). Note the homogeneity of the fiber-type distribution within a given muscle along the trunk. The numbered boxes in e refer to the images in Figure 6. The arrowheads indicate the portion of the M. dorsalis trunci referred to as M. intertransversarius. Pie charts to the right depict the anatomical cross-sectional areas (a-csa) at the respective five cranio-caudal levels a to e of the M. interspinalis (is), M. dorsalis trunci (dt), M. subvertebralis pars lateralis (sl), and M. subvertebralis pars medialis (sm) as percentage of the area of all perivertebral muscles.

to the middle septum, predominantly white fibers occurred while the middle part of the muscle was mainly composed of intermediate fibers (see Fig. 6). Laterally, conterminous with the M. dorsalis trunci, primarily red fibers occurred. Adjacent

to the M. dorsalis trunci, very few, mainly red, fibers were loosely distributed in a thick layer of connective tissue (see Fig. 5). Closer to the attachment sites, the muscle size decreases and the medio-lateral layers became less distinct than at

TABLE 1. Relative anatomical cross-sectional area (a-csa) of the perivertebral musculature in *A. maculatum* and *A. tigrinum* at different cranio-caudal levels along the trunk (a: 2nd, b: 4th, c: 6th, d: 8th, and e: 10th external segment)

		Per unit body mass				Per unit SVL length			
		is	dt	sl	sm	is	dt	sl	sm
<i>A. maculatum</i>	a	18.3	455.6	180.6	93.4	3.8	95.4	37.8	19.6
	b	10.5	419.3	141.5	66.1	2.2	87.8	29.6	13.8
	c	8.2	357.3	203.2	44.8	1.7	74.8	42.6	9.4
	d	24.6	330.2	218.8	27.9	5.2	69.2	45.8	5.9
	e	24.8	343.0	163.3	15.5	5.2	71.8	34.2	3.3
	Mean	17.3	381.1	181.5	49.5	3.6	79.8	38.0	10.4
	SD	6.9	48.2	27.6	27.7	1.5	10.1	5.8	5.8
<i>A. tigrinum</i>	a	12.4	621.7	376.6	147.7	2.2	108.3	65.6	25.7
	b	25.0	640.5	358.9	142.1	4.4	111.5	62.5	24.7
	c	33.2	607.8	429.0	160.3	5.8	105.8	74.7	27.9
	d	31.3	656.0	311.2	183.7	5.5	114.2	54.2	32.0
	e	13.8	622.4	336.0	188.5	2.4	108.4	58.5	32.8
	Mean	23.1	629.7	362.3	164.5	4.0	109.6	63.1	28.6
	SD	8.6	16.8	39.9	18.7	1.5	2.9	7.0	3.3
	Ratio	1.3	1.7	2.0	3.3	1.1	1.4	1.7	2.8

A-csa (mm<sup>2</sup>) was corrected either by body mass (g) or by snout-vent-length (SVL; mm). All values are times 10<sup>-3</sup>. Additionally, the ratio of the mean relative a-csa of *A. tigrinum* to *A. maculatum* is given at the bottom. Muscle abbreviations: M. interspinalis (is), M. dorsalis trunci (dt), M. subvertebralis pars lateralis (sl), and M. subvertebralis pars medialis (sm).

the level of the intervertebral joint. In addition to this medio-lateral regionalization, a shift in the fiber-type composition was observed from deep to superficial muscle areas, partially due to the dorsal tapering of the muscle. When compared with the superficial area, more red fibers were observed in the deeper muscle area near the vertebra (29% vs. 16% at the 4th body segment and 27% vs. 12% at the 8th segment; Fig. 2). This decrease in the red fibers towards the dorsal edge of the muscle was accompanied by an increase in the percentage of white muscle fibers (29 to 41% and 43 to 84%, respectively), though the intermediate fibers were evenly distributed and showed no trend from deep to superficial areas (means 44% and 33% for both segments).

### M. dorsalis trunci

The M. dorsalis trunci forms the bulk of the epaxial muscle mass and is segmented throughout the trunk by myosepta, which attach to the neural spines and the transverse processes of the vertebrae. The myosepta follow a complex course (Nishi, 1916; Francis, 1934; Auffenberg, 1959; Willemse, 1974; Gemballa and Ebmeyer, 2003) due to which several slips of the dorsalis trunci muscle may be visible in the same histological cross-section (e.g., Figs. 2, 4, and 5). Within the muscle and between successive myosepta, the muscle fibers run in a more or less cranio-caudal direction (Nishi, 1916; Francis, 1934; Willemse, 1974). The fibers of the dorsalis trunci muscle are also referred to as intermyoseptal fibers (Auffenberg, 1959).

A separate portion of the M. dorsalis trunci, extending between the transverse processes of

adjacent vertebrae and lying ventral to the main muscle mass and dorsal to the horizontal septum, is sometimes distinguished from the M. dorsalis trunci as M. intertransversarius (Nishi, 1916; Willemse, 1974; Figs. 2, 4a, and 5a, arrow heads) or intervertebral fibers IV-5 and IV-6 (Auffenberg, 1959). Although the M. intertransversarius could easily be identified in the cross-sections of *A. tigrinum*, either due to its own separating tendon sheet (Fig. 2a, arrowheads) or its slightly oblique fiber orientation resulting in an angled sectioning of the fibers (Figs. 2 and 5), it could not be identified consistently in the serial cross-sections of *A. maculatum* (see Fig. 4).

Myoseptal-vertebral fibers described for *Necturus* (Auffenberg, 1959), running at different angles from the transverse processes, the postzygapophysial processes, or the neural arches and spines to the myosepta cranial or caudad the respective vertebra, could not be identified in the histological cross-sections of either *Ambystoma* species. They are most likely subsumed with the deep areas of the M. dorsalis trunci.

The bulk of dorsalis trunci muscle consisted of white muscle fibers in both species. It is covered superficially by a thick layer of mainly red and intermediate fibers accounting together for about 4/5 of the muscle fibers in this clearly distinguished superficial layer (*r*: 55%, *i*: 29% at the 4th segment; *r*: 45%, *i*: 34% at the 8th segment; Figs. 2, 4, and 5). The few widely spaced white fibers in this superficial layer were particularly small in size. Although the thickness of this layer was independent of the cranio-caudal levels in both species, it was somewhat thinner at the dorsal aspect of the body than laterally and was particularly thinner towards

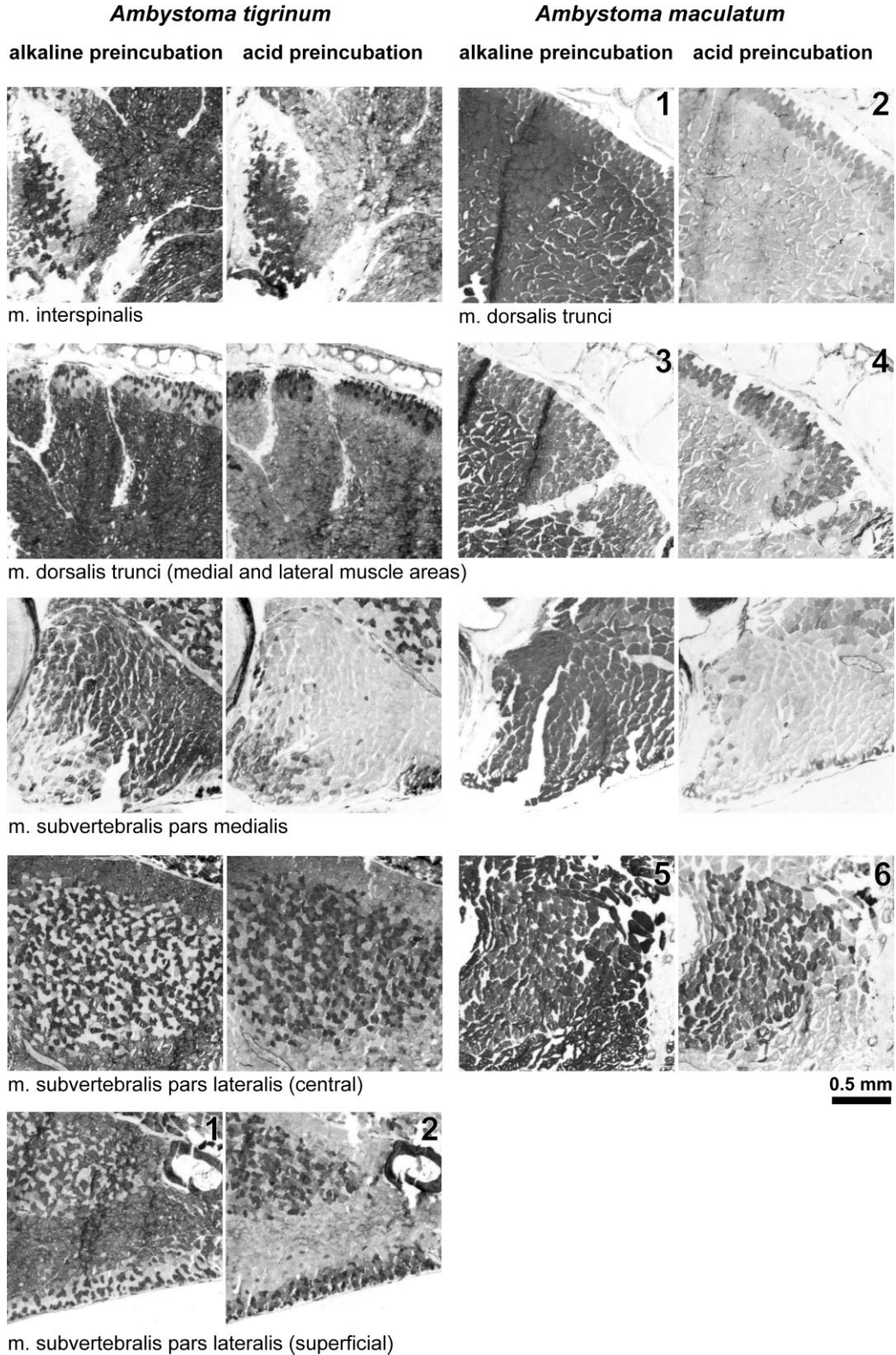


Fig. 6. Selected areas of the perivertebral muscles of both *Ambystoma* species magnified. The numbers in the right top corners refer to the boxed areas in Figures 4 and 5, respectively. The remaining images were taken from the histological cross-sections of the other individual of the respective species. Note the layered fiber-type arrangement in the M. interspinalis, the superficial layer of red and intermediate fibers in the M. dorsalis trunci, the central red/intermediate regionalization in the M. subvertebralis pars lateralis, and the medially higher proportion of red and intermediate fibers in the M. subvertebralis pars medialis.

the myosepta. If the *M. intertransversarius* was distinguishable, it consisted of white muscle fibers; only laterally near the ribs a few red and intermediate fibers were found (Fig. 5e).

### **M. subvertebralis**

The subvertebral muscle makes up the bulk of the hypaxial muscle mass directly lateral and ventral to the vertebral column and passes from vertebra to vertebra ventral to the horizontal septum. Two to three parts are usually distinguished in salamanders.

The pars medialis (Maurer, 1892; Maurer, 1911) attaches to the lateral aspect of each centrum as well as to the ventral face of each transverse process and its fibers run more or less longitudinally (pars subvertebralis according to Francis, 1934). On its lateral edge, it merges, sometimes imperceptibly, into the pars lateralis (Maurer, 1892). The pars lateralis attaches to the ventral and lateral surfaces of the ribs (pars transversalis according to Francis, 1934). The fiber orientation is more oblique than that of the pars medialis (Francis, 1934). A pars obliqua interna, attaching to the myosepta in continuation of the ribs and lying laterally to the pars ventralis, with even more oblique fibers than the pars lateralis, running posterodorsally (Francis, 1934), could be identified in the histological cross-sections of *A. tigrinum* (Fig. 2b, stars), but not consistently in *A. maculatum*. It will be summarized with the pars lateralis herein.

The pars medialis of the *M. subvertebralis* consisted mainly of white muscle fibers in both species (Figs. 2, 4, 5, and 6). Only medially, the proportion of red and intermediate fibers accounted for about 2/3 of the muscle fibers (*w*: 37%, *i*: 28%, *r*: 35% at the 4th body segment and *w*: 35%, *i*: 27%, *r*: 38% at the 8th segment; Fig. 6). Towards the lateral aspect of the muscle, the proportion of the red and the intermediate muscle fibers decreased sharply, so that from at least the middle of the muscle, most of the fibers were white in *A. tigrinum*. In *A. maculatum*, this medial region with a higher proportion of intermediate and red fibers was much smaller and barely noticeable in some sections (e.g., Figs. 4d and 6).

In comparison to the other perivertebral muscles, the *M. subvertebralis* pars lateralis was unusual in having a deep and central region formed by an accumulation of red and intermediate fibers surrounded by purely white fibers in both species. Close to the bone about half of the fibers were white, while the proportion dropped to only 10% in the middle of this region in *A. tigrinum*. The reduction of white fibers was accompanied primarily by an increase in intermediate fibers in both species and the percentage of red fibers maintained more or less the same throughout the central region (mean: 38% at the 4th body

segment, 35% at the 8th segment). The thickness and the distinctiveness of the white area surrounding this central region varied between the two species, being more defined and somewhat thicker in *A. maculatum* when compared with the overall size of the muscle. In result, the central region containing red and intermediate fibers was relatively larger in *A. tigrinum*, particularly in the anterior trunk segments (compare Figs. 4a and 5a). Only in *A. tigrinum*, a superficial layer of red and intermediate fibers occurred towards the more posterior part of the trunk on the ventral aspect of the subvertebral muscle (Figs. 5e and 6).

### **Anatomical Cross-Sectional Area**

The *M. interspinalis* was the smallest muscle of the perivertebral musculature accounting on average for only 2.8% ( $\pm 1.3\%$  SD) of the a-csa in *A. maculatum* and  $1.9 \pm 0.7\%$  in *A. tigrinum*. Its size depended very much on the position of the cross-section relative to its attachments but not the cranio-caudal level (Figs. 4 and 5). The largest muscle is the *M. dorsalis trunci*, which made up almost two thirds of the whole a-csa of the perivertebral musculature ( $60.5 \pm 3.7\%$  in *A. maculatum*,  $53.4 \pm 2.1\%$  in *A. tigrinum*). Neither in *A. maculatum* nor in *A. tigrinum*, a cranio-caudal change was found in the proportion of this muscle (Figs. 4 and 5). Within the *M. subvertebralis*, the lateral part was clearly larger than the medial part in both species (*A. maculatum*:  $29.1 \pm 5.3\%$  vs.  $7.5 \pm 3.6\%$ ; *A. tigrinum*:  $30.7 \pm 2.9\%$  vs.  $13.9 \pm 1.6\%$ ). The ratio between the lateral and the medial part of the *M. subvertebralis* was more or less constant along the body axis in *A. tigrinum*, while the size of the pars medialis decreased caudad in *A. maculatum* (from 12.5% to 2.8%) and the size of the pars lateralis increased (from 24.1 to 29.9%). When both parts were combined, however, the *M. subvertebralis* showed no cranio-caudal change in its percentage of the overall a-csa of the perivertebral musculature in *A. maculatum* as well as in *A. tigrinum*. When compared with the hypaxial muscles, the epaxial muscles accounted for more than half of the a-csa of the musculature (*A. maculatum*:  $63.3 \pm 3.6\%$ , *A. tigrinum*:  $55.4 \pm 2.1\%$ ; Figs. 4 and 5). They were on average only slightly larger than the hypaxials in *A. tigrinum*, but were clearly larger in *A. maculatum* (ratio hypaxials to epaxials: 1.2 and 1.7, respectively). Cranio-caudally, this ratio was constant in both species. Relative to body size, the perivertebral musculature was larger in *A. tigrinum* than in *A. maculatum*, independent of whether it was scaled to body mass or SVL (ratio between species: 1.9 or 1.6, respectively). Within the epaxial musculature, particularly the *M. dorsalis trunci* was larger in *A. tigrinum*, while in the hypaxial muscles the *M. subvertebralis* pars medialis was larger in

*A. tigrinum* than in *A. maculatum* (Table 1). Cranio-caudally, the area of the perivertebral musculature decreased somewhat in *A. maculatum*, while in *A. tigrinum* the areas close to the limb girdles were comparable and slightly larger at mid-trunk (Table 1).

## DISCUSSION

### Fiber-Type Distribution in the Perivertebral Muscles in Salamanders

None of the perivertebral muscles investigated in the two *Ambystoma* species showed a homogeneous fiber-type composition. Rather, all muscles showed a mix of all three fiber types with accumulations of red and intermediate fibers in one and white fibers in another muscle area. Although muscle regions comprising only white fibers occurred in both species, red and intermediate fibers were colocalized in all muscles except the *M. interspinalis*. This positional correlation of red and intermediate fibers has been also described for limb muscles of *Rana esculenta* (Asmussen and Kiessling, 1974). The inhomogeneous fiber-type distribution with regionalizations of a given fiber type implies the existence of functional subunits within the muscles, which possibly function during different tasks as has been shown for fish trunk muscles (e.g., Bone, 1966; Rayner and Keenan, 1967; Ellerby et al., 2000).

The *M. interspinalis* was distinct in its layer-wise organization of the muscle fiber types and its high percentage of red and intermediate fibers. Together with its monosegmental anatomy, its histochemical profile would be well suited to ensure the integrity of the spine and prevent vertebral dislocation. Its bilateral activation pattern during swimming and trotting is consistent with a function as a local stabilizer that is active during both lateral flexion and extension (Deban and Schilling, in press).

In the *M. dorsalis trunci*, the muscle fiber types were arranged in a pattern of red and intermediate fibers occurring in a superficial layer and covering the bulk of the deeper white muscle mass. The same pattern has been described previously for the axolotl *Ambystoma mexicanum* (Totland, 1976a). According to its histochemical profile, the superficial layer is well suited for long-lasting, slow activity, while the main muscle mass is likely to be involved in the production of lateral bending as during locomotion. A muscle activity appropriate for the production of lateral bending has been shown in several salamander species during swimming and trotting (Frolich and Biewener, 1992; D'Aout et al., 1996; Delvolve et al., 1997). Additionally, the *M. dorsalis trunci* was suggested to stiffen the trunk during terrestrial trotting because its activity significantly decreased during aquatic trotting, that is, when the body is buoyed

and gravitational forces are reduced but similar trunk bending amplitudes and frequencies were observed (Deban and Schilling, in press).

Furthermore, the superficial muscle fibers of the *M. dorsalis trunci* have been suggested to maintain tension in the dermis, allowing it to function as a tendon and thus transmit bending forces along the trunk to modulate body stiffness in neotenic *Ambystoma tigrinum* during swimming (Frolich and Biewener, 1992). This proposed function is similar to the mechanics of fish skin (Wainwright et al., 1978). Reorganization of the dermal collagen fibers during metamorphosis into a looser arrangement in adults indicates a reduced function of the dermis in force transmission in adults (Frolich and Schmid, 1991). No study has tracked the muscle-fiber-type distribution during metamorphosis; however, the striking similarity in the fiber-type distribution between the neotenic *Ambystoma mexicanum* (Totland, 1976a; Flood et al., 1977) and the two adult *Ambystoma* species investigated herein points to a conserved pattern of fiber-type distribution and possibly to a similar functionality between neotenic and adult salamanders. The superficial muscle layer may therefore play a role in modulating the body stiffness throughout a salamander's life; in aquatic neotenic individuals via the dermis and the myoseptal system, and in adults primarily via the myoseptal system.

The *M. subvertebralis pars medialis* showed a regionalization in the medio-lateral direction with a decreasing percentage of red and intermediate fibers, while the *M. subvertebralis pars lateralis* contained a central accumulation of these two fiber types surrounded by a thick layer of white fibers. Because of its topography, lying ventro-lateral to the vertebral column, the *pars medialis* is in a good position to sagittally flex the spine but also resist sagging of the trunk, for example, during terrestrial locomotion. The fatigue-resistant medial region of the muscle would be particularly well suited to fulfill an anti-gravity function and especially to locally stabilize the intervertebral joints over longer periods of time. The lateral, fast-twitch region may play a role in lateral bending and/or sagittal flexion given its histochemical profile. The timing of its activity during swimming and trotting supports a function of the *M. subvertebralis pars medialis* in lateral bending and/or sagittal flexion and the significant decrease in activity during aquatic trotting when compared with terrestrial trotting is consistent with this muscle also acting as a stabilizer of the spine (Deban and Schilling, in press).

The *M. subvertebralis pars lateralis*, when compared with the *pars medialis*, has a much better leverage for the production of lateral bending and its activity is appropriate for this (Deban and Schilling, in press). Because its activity also signif-

icantly decreased in aquatic vs. terrestrial trotting, a role in trunk stabilization was discussed. Particularly, the central region with half red and half intermediate fibers accounting for four-fifths of the muscle fibers would be well suited to continually stabilize the body during terrestrial locomotion. Moreover, this muscle region may also be involved in modulating the body's stiffness during swimming. Because connective tissue structures (i.e., dermal collagen fibers and the myoseptal system) are less effective for stiffening the body in salamanders than in fish (Gemballa and Ebmeyer, 2003), muscle action was hypothesized to assist in producing and modulating the body's stiffness (Frolich and Biewener, 1992). The histochemical profile of the central region of *M. subvertebralis pars lateralis* is appropriate for this function.

### Cranio-Caudal Pattern

Along the cranio-caudal axis, no major differences were observed in the salamander's trunk musculature in this study. Neither the fiber-type composition (except the *M. subvertebralis pars lateralis* in *A. tigrinum*) nor the relative size of the muscles changed dramatically in the cranio-caudal direction. Homogeneous muscle properties throughout the trunk were reported for the eel and discussed in relation to its anguilliform swimming pattern (D'Aout et al., 2001). In carangiform fishes, however, clear cranio-caudal changes in the size of the respective muscles and/or the muscle-fiber-type composition were observed (Gill et al., 1989).

The homogenous histochemical profile and muscle size along the salamander's trunk may have several functional interpretations. First, it may reflect a uniform use of the trunk during locomotion with comparable functional demands on each body segment, or it could be related to changing and thus partially conflicting roles of different trunk regions during different locomotor activities, consequently restricting specialization of particular regions. During terrestrial locomotion, maximum lateral excursion occurs around the mid-trunk, while virtually no lateral bending occurs posterior to the shoulder region and at the sacro-caudal region (Roos, 1964; Daan and Belterman, 1968; Frolich and Biewener, 1992). During swimming, salamanders show anguilliform trunk motions with caudad increasing amplitudes (Frolich and Biewener, 1992; D'Aout and Aerts, 1997). Thus, aquatic locomotion may favor uniformity along the trunk or maybe specialization of the posterior region, whereas terrestrial locomotion might favor muscular specialization around the mid-trunk, which undergoes the greatest excursions. Because salamanders have to perform in both terrestrial and aquatic environments, they use their trunk in different ways and this

may hinder specialization of a particular region. Therefore, the uniform histochemical profile and muscle size among the body segments might be related to an amphibious mode of life and comparative studies investigating different ecotypes are necessary to test this.

### Anatomical Cross-Sectional Area

The two species investigated in this study differed in the relative muscle sizes, *A. tigrinum* having larger muscles than *A. maculatum*, a pattern that may be related to differing burrowing behavior of these two species. Particularly, the subvertebral muscle mass was relatively larger in *A. tigrinum* than in *A. maculatum*. Overall, *A. tigrinum* appears more stout and robust than *A. maculatum*. Both species can be considered equally terrestrial and only migrate to ponds or swamps for breeding (Petranka, 1998). *A. tigrinum* lives in burrows but is also active on the surface. Adults actively dig burrows using one forelimb for several quick backward thrusts before the other forelimb takes over. When sufficient soil is loosened, the soil is pushed behind the body with alternating strokes of the hindlimbs (Gruberg and Stirling, 1972). When compared with other *Ambystoma* species, only *A. tigrinum* is capable of digging in soils with very different properties (Semlitsch, 1983a). In contrast, *A. maculatum* spends most of its time underground and does not actively dig; it only enlarges existing holes or cracks with its snout and body (Semlitsch, 1983a).

During digging, extrinsic fore- and hindlimb retractors act on the trunk and produce moments that laterally bend but also sagittally extend and flex the trunk, respectively (Barclay, 1946; Gray, 1968). The topographies of the *M. dorsalis trunci* and the *M. subvertebralis pars lateralis* are appropriate to resist lateral bending and thus could assist other trunk muscles such as the abdominal wall muscles in counteracting lateral bending moments caused by extrinsic fore- and hindlimb muscle action. In accordance, both muscles were larger in *A. tigrinum* than in *A. maculatum*. Furthermore, the *M. subvertebralis pars lateralis* had a relatively greater central region of red and intermediate fibers in *A. tigrinum*, which moreover covered a relatively larger muscle area in the anterior trunk segments. The *pars medialis* of the *M. subvertebralis*, presumably together with other trunk muscles such as the *M. rectus abdominis*, would be in a good position to counteract sagittal extension and to provide local stability of the spine. Consistent with digging behavior, this muscle was clearly larger in *A. tigrinum* and has a relatively greater area of red and intermediate muscle fibers. Furthermore, the relatively larger average size of the *M. interspinalis* in *A. tigrinum* implies increased need for local stability as may occur during digging.

As a caveat, our a-csa results are from a male *A. tigrinum* and a female *A. maculatum* raising the possibility that species differences are confounded by sexual dimorphism. In *A. tigrinum*, breeding females do not significantly differ from breeding males in SVL (Semlitsch, 1983b), while in *A. maculatum*, females are about 10–15% longer (Husting, 1965; Finkler et al., 2003). Thus, if our results were to be biased due to these sex differences, we would expect the female *A. maculatum* to have larger muscles than the male *A. tigrinum*, but we found the opposite. Unfortunately, no study investigated differences in body proportions between sexes, and therefore, no information is available on whether the size differences may affect some body parts more than others. The overall similarity in the fiber-type distribution that we found does not point to large sex differences. However, our data are based on a very small sample size, and more data, that is, more individuals and species, and particularly data on muscle activity during digging are necessary to test the suggested hypotheses.

### Intramuscular Organization in Vertebrate Axial Muscles

Red fibers in urodeles possess many mitochondria, the fewest myofibrils per fiber volume, scanty sarcoplasmic reticulum, multiple innervations, graded contraction and thus correspond to the red, parietal, slow, tonic fibers of other vertebrates, while the white urodelan fibers correspond to the white, central, fast, twitch fibers, and the intermediate fibers of urodeles are similar to intermediate fibers of other vertebrates [e.g., hagfish: (Flood and Storm Mathiesen, 1962; Mellgren and Storm Mathisen, 1966; Dahl and Nicolaysen, 1971); lamprey: (Maurer, 1894; Teräväinen, 1971; Lie, 1974; Meyer, 1979); chondrichthians: (Kryvi, 1977; Kryvi and Totland, 1978); teleosts: (Johnston et al., 1975; Bone, 1989); lungfish: (Dunn et al., 1981); urodeles: (Totland, 1976a,b)].

An intramuscular organization with a central localization of white and a superficial position of red and intermediate fibers was found in the axial muscles of all vertebrates listed above and also observed in *Branchiostoma* (Flood, 1968). It is similar to the pattern described for the *M. dorsalis trunci* in *Ambystoma* (Totland, 1976a; this study). Thus, in their epaxial myomeres, urodeles have retained the plesiomorphic pattern of notochordates (Cephalochordata + Craniata) (Flood et al., 1977; Bone, 1989), although the red and intermediate fibers are intermingled superficially and are not arranged in discrete layers as in other groups (Totland, 1976a). The reversed pattern is observed in amniotes, in which the oxidative fibers are usually found deep in the muscle, close to the vertebral column, while glycolytic fibers are superficial (Schilling, 2009; Moritz, pers. commun.). This pat-

tern is similar to the distribution found in the sub-vertebral muscles in *Ambystoma*. Thus, *Ambystoma* resembles the “fish-like” pattern in its epaxial but the “amniote-like” pattern in its hypaxial muscles.

Accumulations of a certain fiber type in a specific muscle region is a well-known and widespread phenomenon of muscular organization in vertebrates. Various factors have been discussed to account for a certain, “preferred” location of a given fiber type such as thermal balance or mechanical advantage (reviewed in Kernell, 1998). We propose two additional hypotheses can be considered. The change in intramuscular organization during tetrapod evolution may be connected first, to independence from the aquatic environment and second, to the profound reorganization of the axial musculature. In anamniotes superficial red and intermediate fibers attached to the skin play an important role in transmitting bending forces along the trunk during swimming and thus may be an adaptation to life in water (Lie, 1974), however, no muscle fibers attach to the skin in amniotes. Rather, the skin is moveable above the musculature, excluding any function in force transmission. The superficial muscle layer with red and intermediate fibers may, therefore, have been lost during the evolution of the moveable skin of amniotes. With regard to the segmental organization of anamniote muscles, the evolution of polysegmental muscle tracts in amniotes may have increased the importance of local stabilization of the intervertebral joints to allow the polysegmental muscles to act on larger units of the spine without causing intervertebral instabilities. To provide local stabilization and prevent vertebral dislocation, short muscle bundles containing fatigue-resistant fibers must be positioned close to the vertebral column. Thus, architectural constraints and increased needs for local stability may have had an influence on the muscle-fiber-type distribution and the size of the respective muscles. Further research is necessary to increase our understanding of how and why the observed patterns in intramuscular organization evolved in vertebrates, and salamanders with their mosaic muscle pattern can provide further insight.

### ACKNOWLEDGMENTS

We would like to thank the two reviewers, the editor, and R. Salzer for helpful comments on the manuscript. Thanks also to I. Weiß and A. Büscher for technical assistance.

### LITERATURE CITED

- Ashley-Ross MA, Barker PJ. 2002. The effect of fiber-type heterogeneity on optimized work and power output of hindlimb muscles of the salamander *Ambystoma tigrinum*. *J Comp Physiol A* 188:611–620.
- Asmussen G, Kiessling A. 1974. Charakterisierung von besonderen Muskelfasergruppen in der Skelettmuskulatur des

- Frosches durch ihre Innervation und ihre Gefäßversorgung. *Acta Anat* 90:226–242.
- Auffenberg W. 1959. The epaxial musculature of *Siren*, *Amphiuma*, and *Necturus* (Amphibia). *Bull Florida State Mus* 4:251–265.
- Azizi E, Brainerd EL. 2007. Architectural gear ratio and muscle fiber strain homogeneity in segmented musculature. *J Exp Zool* 307A:145–155.
- Barclay OR. 1946. The mechanics of amphibian locomotion. *J Exp Biol* 23:177–203.
- Bennett WO, Simons RS, Brainerd EL. 2001. Twisting and bending: The functional role of salamander lateral hypaxial musculature during locomotion. *J Exp Biol* 204:1979–1989.
- Boisvert CA. 2009. Vertebral development of modern salamanders provides insights into a unique event of their evolutionary history. *J Exp Zool B* 312B:1–29.
- Bone Q. 1966. On the function of the two types of myotomal muscle fibre in elasmobranch fish. *J Marine Biol Ass* 46:321–349.
- Bone Q. 1989. Evolutionary patterns of axial muscle systems in some invertebrates and fish. *Am Zool* 29:5–18.
- Burke RE. 1981. Motor units: Anatomy, physiology, and functional organization. In: Brookhart JM, Mountcastle VB, Editors. *Handbook of Physiology Section I: The Nervous System II Motor Control, Part I, Vol. 10*. Bethesda: American Physiological Society. pp 345–422.
- Carrier DR. 1993. Action of the hypaxial muscles during walking and swimming in the salamander *Dicamptodon ensatus*. *J Exp Biol* 180:75–83.
- D'Aout KD, Aerts P. 1997. Kinematics and efficiency of steady swimming in adult axolotls (*Ambystoma mexicanum*). *J Exp Biol* 200:1863–1871.
- D'Aout KD, Aerts P, De-Vree F. 1996. The timing of muscle strain and activation during steady swimming in a salamander, *Ambystoma mexicanum*. *Neth J Zool* 46:263–271.
- D'Aout KD, Curtin NA, Williams TL, Aerts P. 2001. Mechanical properties of red and white swimming muscles as a function of the position along the body of the eel, *Anguilla anguilla*. *J Exp Biol* 204:2221–2230.
- Daan S, Belterman T. 1968. Lateral bending in locomotion of some lower tetrapods. *Proc Kon Ned Akad Wet (C)* 71:245–266.
- Dahl HA, Nicolaysen KR. 1971. Actomyosin ATPase activity in Atlantic hagfish muscles. *Histochemie* 28:205–210.
- Deban SM, Schilling N. Activity of trunk muscles during aquatic and terrestrial locomotion in *Ambystoma maculatum*. *J Exp Biol* (in press).
- Delvolve I, Bem T, Cabelguen JM. 1997. Epaxial and limb muscle activity during swimming and terrestrial stepping in the adult newt, *Pleurodeles waltl*. *J Neurophysiol* 78:638–650.
- Dunn EA, Davison W, Maloij GMO, Hochachka PW, Guppy M. 1981. An ultrastructural and histochemical study of the axial musculature in the African lungfish. *Cell Tissue Res* 220:599–609.
- Ellerby DJ, Altringham JD, Williams T, Block BA. 2000. Slow muscle function of Pacific bonito (*Sarda chiliensis*) during steady swimming. *J Exp Biol* 203:2001–2013.
- Evans FG. 1946. The anatomy and function of the foreleg in salamander locomotion. *Anat Rec* 95:257–281.
- Finkler MS, Sugalski MT, Claussen DL. 2003. Sex-related differences in metabolic rate and locomotor performance in breeding spotted salamanders (*Ambystoma maculatum*). *Copeia* 2003:887–893.
- Flood PR. 1968. Structure of the segmental trunk muscle in amphioxus. *Cell Tissue Res* 84:389–416.
- Flood PR, Storm Mathiesen J. 1962. A third type of muscle fibre in the parietal muscle of the Atlantic hagfish *Myxine glutinosa*. *Z Zellforsch* 58:638–640.
- Flood PR, Kryvi H, Totland GK. 1977. Onto-phylogenetic aspects of muscle fiber types in the segmental trunk muscles of lower chordates. *Folia Morphol* 25:64–67.
- Francis ETB. 1934. *The Anatomy of the Salamander*. London: Oxford University Press. pp 1–381.
- Frolich L, Biewener AA. 1992. Kinematic and electromyographic analysis of the functional role of the body axis during terrestrial and aquatic locomotion in the salamander *Ambystoma tigrinum*. *J Exp Biol* 162:107–130.
- Frolich LM, Schmid TM. 1991. Collagen type conservation during metamorphic repatterning of the dermal fibers in salamanders. *J Morph* 208:99–107.
- Gemballa S, Ebmeyer L. 2003. Myoseptal architecture of sarcopterygian fishes and salamanders with special reference to *Ambystoma mexicanum*. *Zoology* 106:29–41.
- Gill HS, Weatherley AH, Lee R, Legere D. 1989. Histochemical characterization of myotomal muscle of five teleost species. *J Fish Biol* 34:375–386.
- Gray J. 1944. Studies on the mechanics of the tetrapod skeleton. *J Exp Biol* 20:88–116.
- Gray J. 1968. *Animal Locomotion*. New York: Norton. pp 1–479.
- Gruberg ER, Stirling RV. 1972. Observations on the burrowing habits of the tiger salamander (*Ambystoma tigrinum*). *Herpetol Rev* 4:85–89.
- Husting EL. 1965. Survival and breeding structure in a population of *Ambystoma maculatum*. *Copeia* 1965:352–362.
- Johnston IA, Patterson S, Ward P, Goldspink G. 1974. The histochemical demonstration of myofibrillar adenosine triphosphatase activity in fish muscle. *Can J Zool* 52:871–877.
- Johnston IA, Ward PS, Goldspink G. 1975. Studies on the swimming musculature of the rainbow trout. I. Fibre types. *J Fish Biol* 7:451–458.
- Kernell D. 1998. Muscle regionalization. *Can J Appl Physiol* 23:1–22.
- Kryvi H. 1977. Ultrastructure of the different fibre types in axial muscles of the sharks *Etmopterus spinax* and *Galeus melastomus*. *Cell Tissue Res* 184:287–300.
- Kryvi H, Totland GK. 1978. Fibre types in locomotory muscles of the cartilaginous fish *Chimaera monstrosa*. *J Fish Biol* 12:257–265.
- Lännergren J. 1979. An intermediate type of muscle fibre of *Xenopus laevis*. *Nature* 279:254–256.
- Lie HR. 1974. A quantitative identification of three muscle fiber types in the body muscles of *Lampetra fluviatilis* and their relation to blood capillaries. *Cell Tissue Res* 154:109–119.
- Lutz GJ, Bremner S, Lajevardi N, Lieber RL, Rome LC. 1998. Quantitative analysis of muscle fibre type and myosin heavy chain—Distribution in the frog hindlimb: implications for locomotory design. *J Muscle Res Cell Motil* 19:717–731.
- Maurer F. 1892. Der Aufbau und die Entwicklung der ventralen Rumpfmuskulatur bei den urodelen Amphibien und deren Beziehungen zu den gleichen Muskeln der Selachier und Teleostier. *Morphol Jahrb* 18:76–179.
- Maurer F. 1894. Die Elemente der Rumpfmuskulatur bei Cyclostomen und höheren Wirbeltieren. *Morphol Jahrb* 21:473.
- Maurer F. 1911. Die ventrale Rumpfmuskulatur von *Menobranchnus*, *Menopoma* und *Amphiuma*, verglichen mit den gleichen Muskeln anderer Urodelen. *Jen Zeitschr* 47:1–40.
- Mellgren SI, Storm Mathiesen J. 1966. Oxidative enzymes, glycogen, and lipid in striated muscle. A histochemical study in the Atlantic hagfish (*Myxine glutinosa* L.). *Z Zellforsch* 71:169–188.
- Meyer W. 1979. Oxidative enzymes and myosin-ATPase in the trunk musculature of the river lamprey (*Lampetra fluviatilis*). *Histochem J* 11:187–195.
- Nishi S. 1916. Zur vergleichenden Anatomie der eigentlichen (genuinen) Rückenmuskeln. *Morphol Jahrb* 50:167–318.
- Ogata T, Mori M. 1964. Histochemical study of oxidative enzymes in vertebrate muscles. *J Histochem Cytochem* 12:171–182.
- Petranka JW. 1998. *Salamanders of the United States and Canada*. Washington: Smithsonian Institution Press. pp 1–587.
- Rayner MD, Keenan MJ. 1967. Role of red and white muscles in the swimming of the skipjack tuna. *Nature* 214:392–393.
- Roos PJ. 1964. Lateral bending in newt locomotion. *Proc Kon Ned Akad Wet (C)* 67:223–232.

- Rowlerson AM, Spurway NC. 1988. Histochemical and immunohistochemical properties of skeletal muscle fibres from *Rana* and *Xenopus*. *Histochem J* 20:657–673.
- Schilling N. 2009. Metabolic profile of the perivertebral muscles of small therian mammals: Implications for the evolution of the mammalian trunk musculature. *Zoology* 112:279–304.
- Semlitsch RD. 1983a. Burrowing ability and behavior of salamanders of the genus *Ambystoma*. *Can J Zool* 61:616–620.
- Semlitsch RD. 1983b. Structure and dynamics of two breeding populations of the eastern tiger salamander, *Ambystoma tigrinum*. *Copeia* 1983:608–616.
- Sherkov JK. 1970. Morpho-physiological characterization of peripheral motor apparatus in *Salamandra salamandra*. *J Evol Biochem Physiol* 6:467–469.
- Simons RS, Brainerd EL. 1999. Morphological variation of hypaxial musculature in salamanders (Lissamphibia: Caudata). *J Morph* 241:153–164.
- Smith RS, Ovalle WK. 1973. Varieties of fast and slow extraxial muscle fibres in amphibian hind limb muscles. *J Anat* 116:1–24.
- Snow DH, Billeter R, Mascarello F, Carpen E, Rowlerson A, Jenny E. 1982. No classical type IIB fibres in dog skeletal muscle. *Histochem Cell Biol* 75:53–65.
- Starck D. 1978. Vergleichende Anatomie der Wirbeltiere auf evolutionsbiologischer Grundlage. Bd. 3: Organe des aktiven Bewegungsapparates, der Koordination, der Umweltbeziehung, des Stoffwechsels und der Fortpflanzung. Berlin, Heidelberg, New York: Springer Verlag. pp 1–1110.
- Teräväinen H. 1971. Anatomical and physiological studies on muscles of lamprey. *J Neurophysiol* 34:954–973.
- Tonge DA, Holder N, Jesani P. 1985. Organization of skeletal muscle in the urodele *Triturus cristatus*: Muscle fibre types and motor units. *Proc R Soc Lond B* 223:495–510.
- Totland GK. 1976a. Histological and histochemical studies of segmental muscle in axolotl *Ambystoma mexicanum*. *Norw J Zool* 24:73–84.
- Totland GK. 1976b. Three muscle fibre types in the axial muscle of axolotl (*Ambystoma mexicanum* Shaw): A quantitative light- and electron microscopic study. *Cell Tissue Res* 168:65–78.
- Wainwright SA, Vosburgh F, Hebrank JH. 1978. Shark skin: Function in locomotion. *Science* 202:747–749.
- Willemse JJ. 1974. Arrangements of connective tissue fibres and muscle fibres in the tail musculature of adult newts (*Triturus cristatus*, *T. alpestris* and *T. vulgaris*) (Amphibia, Urodela). *Zoomorphology* 77:255–269.
- Worthington RD, Wake DB. 1972. Patterns of regional variation in the vertebral column of terrestrial salamanders. *J Morph* 137:257–277.