

Morphological and histochemical characterization of the pectoral fin muscle of the stripped weakfish, *Cynoscion guatucupa*

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Introduction

The muscles that move the pectoral fin of teleosts derive from the paraxial mesoderm, specifically from the dermomyotome. The pectoral muscles comprise two antagonistic masses: lateral or dorsal, composed of abductors (extensors) muscles; and medial or ventral, composed of adductor (flexor) muscles. These muscles originate mainly in the cleithrum and in two other associated bones, the coracoid and the scapula; they insert in the radials or in the base of the fin rays. The position of the pectoral fins is correlated with the evolutionary position of a fish species; generally, more derived fishes possess fins located higher on the body, allowing more active swimming

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Abstract

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The fibres of superficial and deep abductor muscles of the pectoral fins of the stripped weakfish, *Cynoscion guatucupa* have been studied using histochemical techniques: succinic dehydrogenase (SDH) for mitochondria, periodic acid–Schiff (PAS) for glycogen, myosin-adenosin triphosphatase (mATPase) to identify different fibre types based on the contraction speed and modified ATPase to identify capillaries. The fibre diameters were measured, and the capillaries of the main fibre types – red, pink and white— were counted. The two muscles showed both macroscopically and microscopically two well-differentiated zones with predominant white fibres. The area of insertion of muscles into the fin rays had red, pink and white fibres. The origin zone of the muscle into the bone was composed by white fibres only. Both zones of white muscle evidenced a mosaic of small, medium and large polygonal white fibres. Red, pink and white muscles showed a wide histochemical diversity of fibre subtypes. The area per peripheral capillary increased from the red to the white muscles. Due to the predominance of white fibres, the pectoral fins of *C. guatucupa* were mainly involved in rapid movements to stop/discontinue and stabilize the body during swimming.

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behaviours. The possible movements are diverse: abduction, adduction, lean position and even convolution (Winterbottom 1974; Cousseau 2010).

The myotomal organization of the teleost musculature reveals three different types of fibres: (i) red muscle, superficially located; (ii) white muscle, deeply located; (iii) pink muscle, located between the red and white muscles. There are vast interspecies differences in terms of the proportion of these muscle types to each other and their histochemical and biochemical properties (Devincenti *et al.* 2000). However, in the fin muscles, a characteristic and complex morphological distribution of different types of fibres has been found (Chayen *et al.* 1987; Devincenti *et al.* 2009).

In Antarctic fishes with labriform swimming, numerous studies have found that the pectoral fins are mainly used

for locomotion and the myotomal musculature gives rigidity to the body (Walesby and Johnston 1980; Davison and MacDonald 1985; Johnston 1989; Sanger *et al.* 2005).

However, few studies have examined the fin musculature of fishes with a subcarangiform mode of swimming, which results in more undulations along the posterior part of the body. These fishes employ their pectoral fins in breaks, stabilization and turning manoeuvres (Helfman *et al.* 2009).

The stripped weakfish, *Cynoscion guatucupa* is one of eight species of the genera *Cynoscion* described by Cousseau and Perrotta (2000) for the South American Atlantic coasts; it belongs to the family Scianidae and lives from 22°S 35' in Rio de Janeiro, Brasil, to 43°S in Argentina. This species, like the whitemouth croaker *Micropogonias furnieri*, displays a subcarangiform swimming pattern. We have analysed the abductor muscle of pectoral fins of *M. furnieri*. We found three zones in the pectoral musculature: superficial, medial and deep, with red, pink and white fibres, respectively (Devincenti *et al.* 2009). Even though the histochemical and ultrastructural studies on myotomal musculature of *C. guatucupa* have been carried out (Devincenti *et al.* 1998), no histochemical information is available at present for the characterization of the fibre types in the pectoral fins of this highly prized commercial species.

The purpose of this work is to analyse the morphology, histochemistry and morphometry of the abductor muscle of the pectoral fins of *C. guatucupa*. A second goal is to find possible relationships between the muscle fibre composition and the pectoral fin functions, such as swimming behaviour.

Materials and Methods

Animals

The mature female specimens ($n = 9$; length 47.5 ± 4.0 cm) of *C. guatucupa* used in this research were obtained from the commercial and sport fishery in the city of Mar del Plata (Argentina). Individuals were sacrificed by cervical dislocation after the guidelines of the American Fisheries Society (AFS 2004). The following pectoral muscles were dissected and anatomically described: superficial and deep abductor and arrector ventralis. The superficial and deep abductor muscles were used for histochemical and morphometric analyses.

Histochemical methods

Blocks of tissue were frozen by immersion in liquid nitrogen for 60 s, stored at -25° C and examined during the following 4 weeks. Ten- to 12- μ m-thick frozen sections were cut, mounted on glass slides and stained with the following histochemical techniques:

1. Periodic acid–Schiff (PAS) method (Hotchkiss 1948): for glycogen detection. Controls were made with α -amylase.

2. Succinic dehydrogenase (SDH) activity (Deffendi and Pearson 1955): to detect the oxidative capacity of the fibres. The activity of this enzyme was demonstrated using the nitroblue tetrazolium technique; controls were conducted by adding sodium malonate as an inhibitor.
3. Myosin-adenosine triphosphatase (m-ATPase; Guth and Samaha 1970): to identify different fibre types based on the contraction speed. A modified test adapted to fishes was used (Devincenti 1998). Sections were preincubated at room temperature at a pH serial range of 4.3–10.6 for various periods; controls with sodium glycerophosphate in the place of substrate were carried out.
4. The mATP-ase-modified technique (Rosenblatt *et al.* 1987): to identify capillaries. This technique was adapted for fishes: incubation at room temperature and material fixation in 5% formaldehyde, 0.36 M sucrose and 0.068 M CaCl_2 at room temperature and at 4° C from one to 5 min (Devincenti 1998).

Measurement of the fibre size

Cross-sections of four individuals were used for this study. The diameters of 100 red, pink and white fibres per animal were, respectively, measured at $312.5\times$, $125\times$ and $102\times$ magnifications in sections stained for m-ATPase (te Kronnie *et al.* 1983). Fibre areas were calculated through the following formula (Alnaqeeb and Goldspink 1986):

$$A = (D/2)^2 \times (\pi)$$

where A = area of a fibre type, and D = mean diameter of a fibre type.

Capillaries

To determine the number of capillaries vascularizing the red, pink and white muscle fibres, 100 fibres of each type were analysed in four specimens. The white muscle of the origin zone was used for this study because its capillaries were more representative than those of the insertion zone. For each fibre type, the percentage of fibres surrounded by 0, 1, 2, 3, 4 and 5 capillaries was calculated. The average number of peripheral capillaries (PC) per fibre type was also obtained. With the PC and the area of each fibre type, the fibre area per peripheral capillary (APC) was calculated (Mosse 1979):

$$APC = A/PC$$

Statistical analysis

Statistical analysis was performed with the Sigma Stat 3.5 program. Mean diameters and number of capillaries between the different fibre types were compared using the Kruskal–Wallis one-way analysis of variance test. Comparisons between pair

of samples were carried out by the Mann–Whitney *U*-test (Zar 2010).

Results

Anatomy

Compared with *M. furnieri*, the pectoral muscles of *C. guatucupa* (Fig. 1A) were characterized by its soft appearance. In this species, the abductor superficialis was a large muscle that pulls the fin forward and away from the body. It originated on the medial/superior part of the cleithrum and inserted via tendons to the anterior bases of the fin rays near radials (Fig. 1B). Macroscopically, it was characterized by the presence of white muscle (proximate to the origin) and red muscle (proximate to the insertion). The abductor profundus and ar-

rector ventralis were observed under abductor superficialis (Fig. 1C). The abductor profundus, smaller than the abductor superficialis, originated in the coracoid and cleithrum bones and inserted via tendons on the ventral rays of the pectoral fin; it showed a macroscopic arrangement of white and red fibres similar to that of the abductor superficialis. The arrector ventralis was the smallest of the three muscles studied, composed basically of white and pink fibres; it spreads the rays to draw the first ray forward and downward. It originated on the cleithrum and inserted via tendons on the base of the first fin ray.

Histochemistry

The superficialis and profundus abductor muscles were separated by a thick connective tissue fascia, the epimysium, with

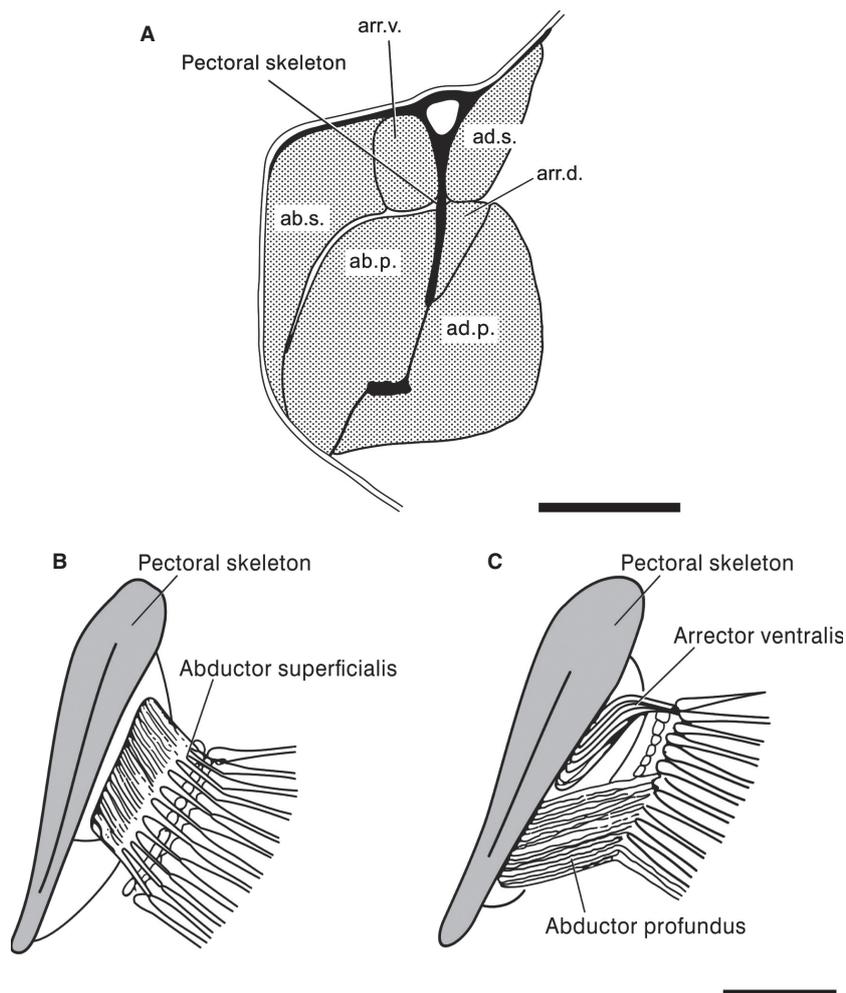


Fig. 1—Diagram of the pectoral fin musculature of *Cynoscion guatucupa*. – **A**. Cross-section through the pectoral muscle mass showing the positions of the muscles – **B**. Lateral view showing the abductor superficialis. – **C**. Lateral view without the abductor superficialis. The abductor profundus and the arrector ventralis are evident. ab.s, abductor superficialis; ad.s, adductor superficialis; ab.p, abductor profundus; ad.p, adductor profundus; arr.d, arrector dorsalis; arr.v, arrector ventralis. Scale bars: 1 cm

abundant blood vessels and nerves. Both muscles could be differentiated macroscopically and microscopically into two well-defined zones: the insertion zone of the muscle into the fin rays, with red, pink and white fibres (Figs 2A–C and 3A), and the zone comprising the origin of the muscle in the bone, with mosaic aspect white fibres (Fig. 2D).

The red fibres measured $32.34 \pm 11.77 \mu\text{m}$ (range 12–72 μm ; Fig. 2A). The pink fibres, located among the red and white ones, were observed in a lesser proportion than the other two fibre types: $65.70 \pm 24.02 \mu\text{m}$ (range 20–122 μm) mean diameter (Fig. 2B). The white fibres comprised a greater proportion of the superficial and profundus abductor muscles. At least two zones were differentiated in the white muscle: proximal, which started next to the pink muscle in the region of insertion, with $57.58 \pm 32.4 \mu\text{m}$ (range 14–172 μm) mean diameter (Fig. 2C), and distal, with $92.66 \pm 47.92 \mu\text{m}$ (range 26–262 μm) fibre mean diameter in the origin region (Fig. 2D). Significant differences were found between the diameters of the red and pink fibres ($P < 0.001$), between the red and white fibres of the two zones ($P < 0.001$), between the pink and white fibres in the insertion zone ($P < 0.001$), between the pink and white fibres in the zone of origin ($P < 0.005$), and between the white fibres of the two zones ($P < 0.001$).

Red fibres resulted in weak to moderate activity both with non-pre-incubated mATPase and pre-incubated at pHs 4.3 and 10.6; they showed greater activity pre-incubated at pHs 4.6, 9.8 and 10.4; they were highly SDH and PAS positive (Table 1). Three different red fibre subtypes were identified: (i) small red fibres (sr), $13.33 \pm 3.35 \mu\text{m}$ (range 5–21 μm) mean diameter, present in a minor proportion, varying enzyme activity (Fig. 3B,C) and glycogen content (Fig. 4C); (ii) large red fibres, so-called ‘other red’ (or), with characteristics similar to those of pink fibres (Figs 3B, C and 4A, C); (iii) standard red fibres (str), comprising most of the fibres, described as a mosaic by PAS and mATPase with pre-incubation at alkaline pHs (Figs 3 B, C and 4A, C).

Pink fibres were histochemically classified into: small pink (sp), which had an mATPase high activity at alkaline pHs (Fig. 3B) and at pH of 4.6, rendered by SDH and PAS positive (Fig. 4B, D); medium pink (mp), which had lower activity for all techniques; and large pink (lp), located among the white fibres, negative for PAS and SDH, had a greater mATPase activity at alkaline pHs (Fig. 3C; Table 1).

In both zones of white muscle, a mosaic of small, medium and large white polygonal fibres was observed (Table 1). Moreover, these fibres revealed a mosaic with mATPase at alkaline pre-incubations (Figs 3D and 5A) and at pH 4.6 (Fig. 5C), SDH (Fig. 4B) and PAS (Fig. 4D); however, the muscle was more homogeneous at pH 4.3 (Fig. 5B). The small white fibres (sw) had the greatest activity for all the techniques (Figs 3D, 4B, D and 5; Table 1). In the origin zone with mATPase, PAS and ATPase for capillaries, at least two fibre regions, were identified with different morphological distributions and enzyme activities (Fig. 5A, B).

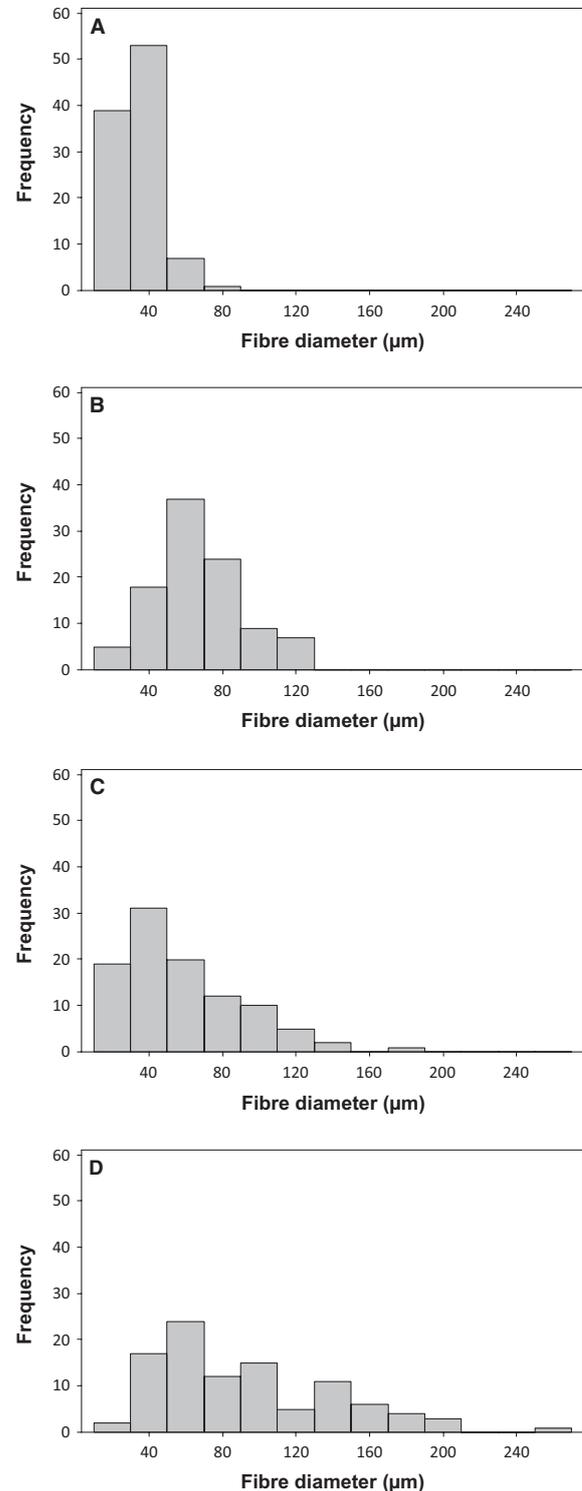


Fig. 2—Frequency histograms of fibre diameters within the different types of fibres of the abductor fin muscle of *Cynoscion guatucupa*. — **A**. Red fibres. — **B**. Pink fibres. — **C**. White fibres of the insertion zone. — **D**. White fibres of the origin zone.

Table 1 Histochemistry of the pectoral fin abductor muscle of *Cynoscion guatucupa*

	Red fibres			Pink fibres			White fibres		
	sr	str	or	s	m	L	s	m	L
PAS	0/3	1/3	0/1	0/2	0/1	0	1/2	0/1	0
SDH	2/3	4	3	1/3	2/3	0/1	2	1/2	0/1
mATPase	1/2	0/1	4	4	3/4	0/2	3	2/3	1/2
No pre-incub									
mATPase	3/4	0/3	4	4	1/4	0/4	2 o	2/3 o	3 o
pH 9.8							1/4 i	0/2 i	3/4 i
mATPase	3	1/3	4	4	4	4	2 o	2/3 o	3 o
10.4							1/4 i	0/3 i	3/4 i
mATPase	1	0/1	4	2/3	2/3	0/4	2 o	2/3 o	3 o
10.6							0/2 i	0/2 i	3 i
mATPase	4	1/4	4	4	0/4	0/1	3	1/2	1
4.6									
mATPase	1/2	0/1	0	0/1	0/1	0	1	0	0
4.3									

Reactivities: (0): negative; (1): weak; (2): moderate; (3): strong; (4): very strong. i, white muscle insertion zone; L, large fibres; m, medium fibres; mATPase, myosin-adenosintriphosphatase; o, white muscle origin zone; or, other red fibres; PAS, Periodic Acid–Schiff; s, small fibres; SDH, succinate dehydrogenase; sr, small red fibres; str, standard red fibres.

Capillaries

The average number of capillaries surrounding a red muscle fibre was 1.99, and the fibre percentage with no peripheral capillaries was 10%. White fibres with 1.22 average capillaries per fibre were less vascularized than the red and pink fibres (Fig. 6). There were a maximum of three capillaries, but 23% of the fibres lacked capillaries. The pink fibres were found vascularized in between the red and white fibres (Fig. 6) and had 1.67 capillaries per muscle fibre (Table 2). Table 3 displays the standardized data for a fibre mean area. The area per peripheral capillary increases from the red to the white muscle. Significant differences ($P < 0.05$) were found between the capillarization of red and white fibres, and pink and white fibres; however, no significant differences ($P < 0.05$) were found between the red and pink fibres (Tables 2 and 3).

Discussion

The anatomical organization of the pectoral abductor muscles of *C. guatucupa* is typical of the structural plan of teleosts. Thus, three abductor muscles were found: abductor superficialis, abductor profundus and arrector ventralis; the first two propel the fin away from the body, and the latter shifts the first fin ray outwards (Cousseau 2010).

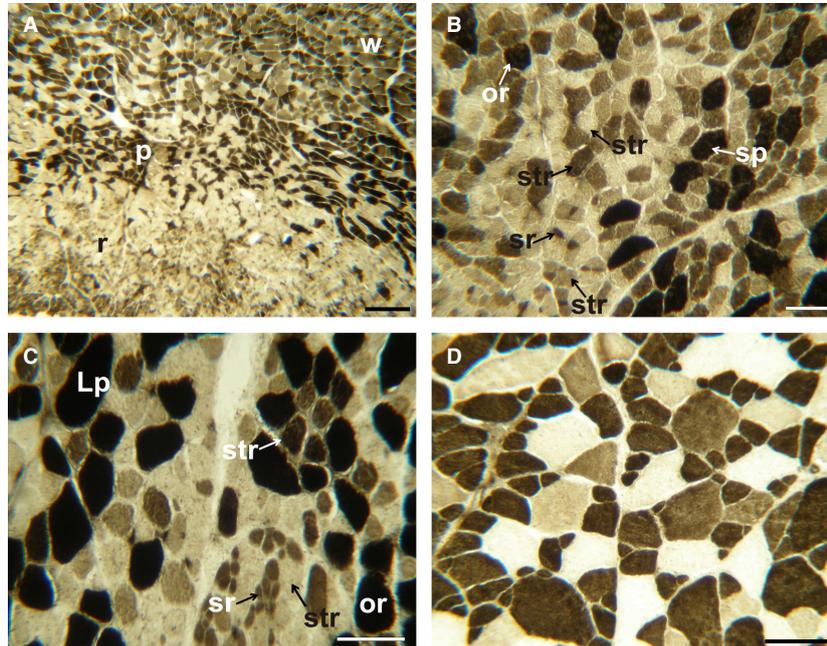


Fig. 3—Histochemical staining of m-ATPase activity in the abductor fin muscle of *Cynoscion guatucupa*. Insertion zone. – **A**. m-ATPase activity with pre-incubation at pH 9.8 showing white (w), pink (p) and red (r) fibres. – **B**. m-ATPase activity without pre-incubation. Red and pink fibres are present. – **C**. m-ATPase activity with pre-incubation at pH 9.8. Pink fibres react strongly to this technique, whereas the red fibres show a mosaic pattern of staining intensities. – **D**. m-ATPase activity with pre-incubation at pH 10.4. A mosaic of white fibres with variable activity is evident. Lp, large pink fibres; or, other red fibres; sp, small pink fibres; sr, small red fibres; str, standard red fibres. Scale bars: – **A** 200 μm , – **B–D** 100 μm .

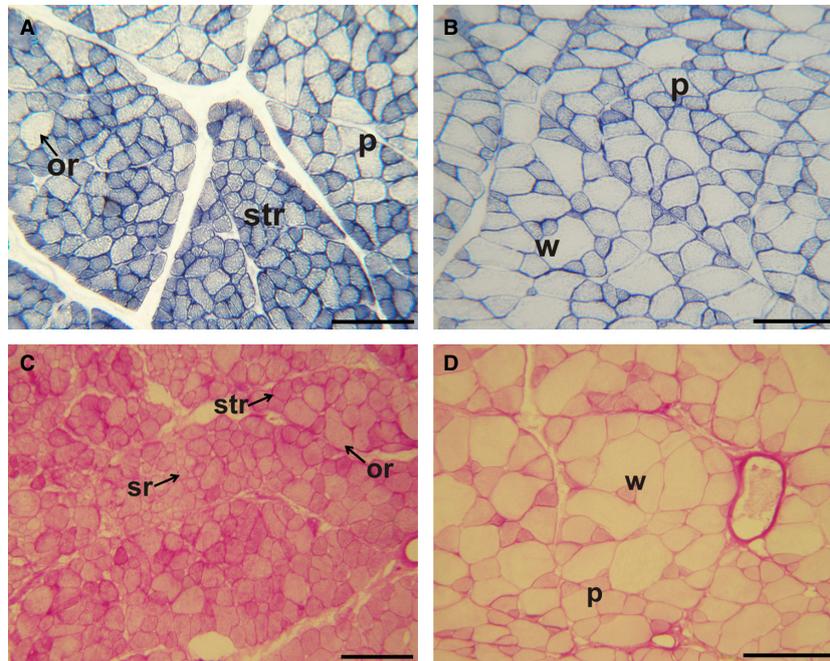


Fig. 4—Histochemical staining of the abductor fin muscle of *Cynoscion guatucupa*. Insertion zone. – **A**. The red and pink (p) fibres show a mosaic of staining intensities with succinic dehydrogenase (SDH) technique. – **B**. SDH activity is moderate in some pink and white (w) fibres, whereas other ones react weakly to SDH. – **C**. periodic acid–Schiff (PAS) showing some red fibres with moderate stain intensity and other ones with strong stain intensity. – **D**. PAS. The white and pink fibres exhibit a mosaic of staining intensities with this technique. or, other red fibres; sr, small red fibres; str, standard red fibres. Scale bars: 100 μ m.

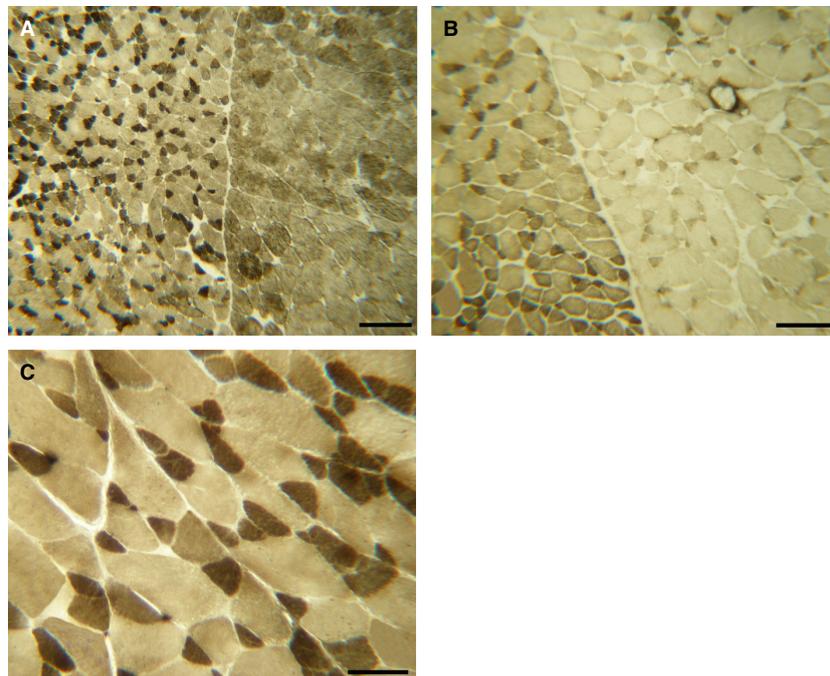


Fig. 5—Histochemical staining of m-ATPase activity in the abductor fin muscle of *Cynoscion guatucupa*. Origin zone. – **A**. m-ATPase activity with pre-incubation at pH 10.6, and – **B**. m-ATPase activity after pre-incubation at pH 4.3. Two different regions of white fibres are present: a mosaic of fibres with variable activity (left side) and a more homogeneous zone (right side). – **C**. m-ATPase activity with pre-incubation at pH 4.6. Detail of the mosaic of white fibres. Scale bars: – **A–B** 200 μ m, – **C** 100 μ m.

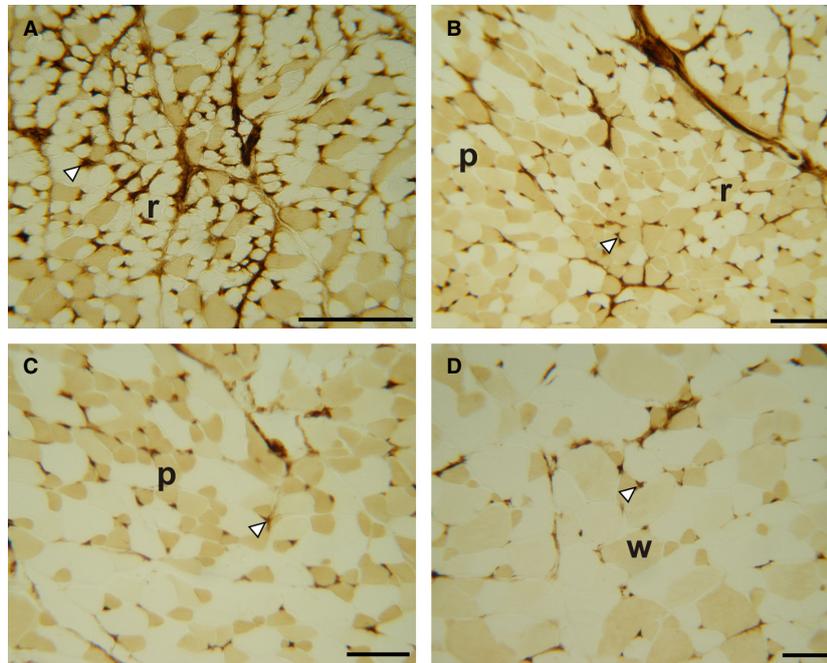


Fig. 6—Histochemical staining of ATPase for capillaries of the abductor fin muscle of *Cynoscion guatucupa*. – **A**. The number of capillaries (white arrow) in red fibres (r) is greater than those found in the other types of pectoral fibres. – **B** and – **C**. The pink fibres (p) exhibited an intermediate irrigation. – **D**. White fibres (w) have the least number of capillaries. Scale bars: 100 μm .

Table 2 Capillary supply of the pectoral fin abductor muscle of *Cynoscion guatucupa*

Fibre	Percentage of fibres surrounded by 0-5 capillaries						PC
	0	1	2	3	4	5	
Red	10	23	32	28	7		1.99 ^a
Pink	21	21	33	20	4	1	1.67 ^a
White	23	40	31	6			1.22 ^b

PC: peripheral capillary average. Similar superscripts express no statistically significant differences ($P > 0.05$). Different superscripts indicate statistically significant differences ($P < 0.05$).

Table 3 Standardized data of capillarization for the mean transverse area of each muscle fibre type in the pectoral fin abductor muscle of *Cynoscion guatucupa*

Fibre	D (μm)	A (μm^2)	PC	APC (μm^2)
Red	^a 32.3 \pm 11.77	821	1.9	432.1
Pink	^b 65.7 \pm 24.02	3388.4	1.67	2028.98
White	^c 92.66 \pm 47.92	6739.91	1.2	5616.59

A, area per muscle fibre type; APC, area per peripheral capillary; D, mean diameter; PC, peripheral capillary average. Similar superscripts express no statistically significant differences ($P > 0.05$). Different superscripts indicate statistically significant differences ($P < 0.05$).

The histochemical techniques used in our study revealed that the superficialis and profundus abductor muscles possess similar characteristics. The white muscle was found in the great-

est proportion, just like it was observed in the myotomal musculature of this species (Devincenti 1998; Devincenti *et al.* 1998).

As demonstrated in this work, different zones were found in the abductor fin muscle of *M. furnieri*: superficial, medial and deep, with red, pink and white fibres, respectively (Devincenti *et al.* 2009), which correspond to the insertion zone of *C. guatucupa* pectoral muscle.

As reported for the myotomal musculature of the species (Devincenti *et al.* 1998), the standard red fibres (str) were the most abundant of the three different red muscle subtypes here characterized. Antarctic fishes, such as *Notothenia rossi* (Walesby and Johnston 1980), *Pogothenia borchgrevinkii* and *Trematomus bernacchii* (Davison and MacDonald 1985), possess pectoral muscles with predominant red fibres and an aerobic capacity greater than that of the myotomal fibres; these facts have been ascribed to the fundamental use of pectoral fins in labriform swimming. Two different red fibre types

appear in *N. rossii* (Walesby and Johnston 1980) and three in the pectoral muscle of *Eleginops maclovinus* (Fernández *et al.* 1999). Nevertheless, red fibres of fishes, such as *M. furnieri*, *Mugil cephalus*, *Cyprinus carpio* and *C. guatucupa*, develop an enzymatic activity equal or less than that of the myotomal muscle, compatible with their subcarangiform swimming mode, where the myotomal musculature plays the main role in swimming (Urfi and Talesara 1989; Langfeld *et al.* 1991; Chayen *et al.* 1993; Devinenti 1998; Devinenti *et al.* 1998, 2000, 2009).

The small red fibres (sr) were found in the pectoral musculature as well as in the myotomes of *C. guatucupa* and *M. furnieri* (Devincenti *et al.* 1998, 2000, 2009). Small fibres of varied position have been described in numerous teleosts from different habitats and systematic locations: some under the skin, some others in between red and pink fibres, and still others in the absence of red fibres, they border on the white ones (Johnston *et al.* 1974; Carpené *et al.* 1982; Matsuoka and Iwai 1984; Sängner 1997). They have also been found in Antarctic fishes that swim by pectoral fins movements (Walesby and Johnston 1980; Davison and MacDonald 1985; Johnston 1989; Fernández *et al.* 2000, 2005). Many are the histochemical, immunohistochemical and ultrastructural studies reporting small red fibres (sr) with features characteristic of tonic fibres in both the myotomal musculature and fins (Karasinsky and Kilarski 1989; Calvo and Johnston 1992; Chayen *et al.* 1993; Sängner 1997; Ramírez-Zarzosa *et al.* 1998; Fernández *et al.* 2000; Martínez Ibabe *et al.* 2000; Sängner and Stoiber 2001; Silva *et al.* 2008; Fernández and Calvo 2009).

The third type of red fibres, the ‘other red’ (or), was also found in the myotomal muscle of *C. guatucupa* (Devincenti *et al.* 1998) and in the myotomal and pectoral musculature of *M. furnieri* (Devincenti *et al.* 2000, 2009), exhibited histochemical characteristics similar to those of pink fibres. This was described too for other teleosts such as *M. cephalus* L., *Gadus virens*, *Hemiramphus melanochir* and *Gymnapistes marmoratus*, and the fibres were then called ‘other red’ (Johnston *et al.* 1974; Mosse and Hudson 1977). Rowleron *et al.* (1985), Ramírez-Zarzosa *et al.* (1998) and Martínez Ibabe *et al.* (2000) described them as infiltrated pink fibres.

With reference to size, histochemical and immunohistochemical properties, pink muscles present large variations between species; even though it is always easily distinguishable from the red muscle, characteristics similar to the white muscle can make it difficult to identify (Mosse and Hudson 1977; Carpené *et al.* 1982). Based on the mATPase and PAS, two different pink fibre zones were determined in the myotomal musculature of *C. guatucupa*: homogeneous superficial pink muscle and mosaic deep pink muscle (Devincenti *et al.* 1998). Although in the pectoral fins of *C. guatucupa* these two zones are not clearly evident, histochemical and morphological differences could be established in two sectors: one, with pink fibres mixed with red ones, which histochemical activity was more homogeneous (histochemical and morphological

features of ‘other red’ fibres); and a second zone, with medium and large size fibres that intermingle with white fibres, with heterogeneous histochemical activity. In labriform swimming notothenioids, the pink fibres are arranged in a mosaic among the red fibres, having an intermediate SDH and glycogen activity, and not differing from the myotomal pink fibres in relation to its mATPase activity (Fernández *et al.* 1999, 2000).

White fibres from both the insertion and origin of the abductor muscle of *C. guatucupa* are classified as mosaic muscles due to the reacting histochemical variation between small, medium and large fibres. Two zones were found in the white myotomal musculature of *C. guatucupa*: a superficial one, with small and medium-sized fibres, and a deep one, with medium-sized and large fibres, with diverse oxidative activity, contracting speed (mATPase) and glycogen content (Devincenti *et al.* 1998). In the abductor pectoral muscle of *M. furnieri*, a mosaic of white fibres were found with histochemical and morphological features correspondent to the white fibres from the insertion zone of *C. guatucupa* (Devincenti *et al.* 2009).

Many teleosts exhibit an indeterminate growth pattern, increasing both their body size and muscular mass until senescence or death (Johnston *et al.* 2011). Thus, the pectoral and myotomal muscles grow by hyperplasia (Patterson *et al.* 2008; Silva *et al.* 2008) as well as hypertrophy (Priester *et al.* 2011). The presence of a histochemical and morphometric mosaic has been related to hyperplastic growth periods during the postlarval stages. New cells originate either from satellite cells (Johnston 1982) or through division of adult fibres (Willemsse and Van De Berg 1978). Martínez Ibabe *et al.* (2000) found mosaic myotomal muscle in 18 of 19 of the species of teleosts analysed, and they concluded that no relation existed between its presence and the seasonal growth; rather, its presence would depend on the species.

The three types of pectoral muscle fibres present histochemical and morphological characteristics similar to their corresponding myotomal muscle. Because of the predominance of white fibres, pectoral fins of *C. guatucupa* could be mainly implicated in rapid movements to discontinue and stabilize the body during subcarangiform swimming. In the other hand, the predominance of white muscle fibres in the myotomal musculature of this species is related to the fast oscillations and undulations of the body during this mode of swimming.

The anatomical, morphometric and histochemical study of the abductor muscle of the pectoral fins of *C. guatucupa* provides morphological basis to the histophysiology of swimming in fishes. Moreover, this work contributes to understanding the general biology of this species.

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