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Preliminary Study of the Sonic Muscle of *Micropogonias furnieri* (Actinopterygii, Sciaenidae): Morphology and Histochemistry

Estudio Preliminar del Músculo del Sonido de *Micropogonias furnieri* (Actinopterygii, Sciaenidae): Morfología e Histoquímica

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SUMMARY: The present study highlights the morphology of the *Micropogonias furnieri* sonic muscle by means of histochemical techniques, relating it to previous histochemical studies of the pectoral fin and myotomal muscles. In order to classify the muscle fibres, succinic dehydrogenase (SDH) for mitochondria, periodic acid Schiff (PAS) for glycogen, Sudan Black and Red for lipids and myosin adenosine triphosphatase (m-ATPase) pre-incubated at alkaline and acid pHs to visualize the contraction velocity, were used. The sonic fibres were smaller than the white myotomal and pectoral fibres, showed homogenous size and distribution and had features common to white fibres: negative to SDH and lipids, weakly positive to PAS and m-ATPase following acid pre-incubation, and positive to m-ATPase at alkaline pre-incubation. The morphology of the sonic muscle of *M. furnieri* showed no differences between sexes, probably due to the fact that the individuals were at the post spawning maturity stage. This similarity would indicate a similar sound production in both sexes, related to the “disturbance calls” of this species.

KEY WORDS: Sonic muscle; *Micropogonias furnieri*; Morphology; Histochemistry.

INTRODUCTION

Fishes show different ways of producing sounds: by rubbing their pharyngeal teeth, through the swim bladder and the sonic muscle, which muscle fibres are striated, and through rapid changes in the velocity and direction of the fish while swimming (hydrodynamics).The muscle joined to the swim bladder, the sonic muscle, and associated structures often appear throughout the evolution of teleosts. The sonic muscle can be intrinsic, joined to both sides of the swim bladder, or extrinsic, originated over an independent bony structure with an insertion into the swim bladder (Parmentier *et al.*, 2006). The sonic muscle can also be used in defence or aggression, for the echolocation of the seabed, or for the formation of shoals of fish. Thus, most of the sound produced would participate in the intraspecific social behaviour and in the interspecific communication (Helfman *et al*., 2009). In the sciaenids, the sonic organ is made up of a swim bladder and two typically extrinsic lateral muscles lying on the wall of the body surrounding the bladder (Ladich & Fine, 2006).

The whitemouth croaker, *Micropogonias furnieri* Desmarest, is a teleost that belongs to the family Sciaenidae, order Perciformes; it shows a wide distribution, from Península de Yucatán (México) to 41° S (Argentina) (Isaac-Nahum, 1988). A marine estuarine fish, *M. furnieri* is a slow growing fish, reaching its maximum size at approximately 70 cm. It feeds mainly on benthic invertebrates and to a lesser extent on small fishes (Cousseau & Perrotta, 2000).

Several aspects of its biology have been studied, some of them dealing with systematics (Díaz de Astarloa & Ricci, 1998), genetics (Pereira *et al*., 2009), reproductive biology (Macchi & Christiansen, 1992; Jaureguizar *et al.*, 2008), general morphology (Figueroa, 1985), morphology and

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histochemistry of the digestive tract and gills (Díaz *et al.*; 2005, 2008) and histochemical analysis of the myotomal and pectoral fin muscles (Devincenti *et al*., 2000; 2009).

M. furnieri produces two different sounds using extrinsic sonic muscles: (1) male advertisement calls during the spawning season and (2) disturbance calls, produced by both sexes (Tellechea *et al*., 2010).

In spite of the aforementioned studies, the morphology and histochemistry of the sonic muscle of *M. furnieri* are still unknown. Therefore, morphological and histochemical studies of sonic muscles in males and females of *M. furnieri* will contribute to the understanding of the mechanism of sound generation in this species.

The present study highlights the morphology of the *M. furnieri* sonic muscle by means of classical and specific histochemical techniques, relating it to previous histochemical studies of the pectoral fin and myotomal muscles.

MATERIAL AND METHOD

Five male and five female adults of *M. furnieri* (total length 47.5 ± 4.0 cm) obtained from commercial and sport fisheries in the coast of Mar del Plata, Argentina (38º 05' S, 57º 32' W) were sacrificed by cervical dislocation. The handling, collection and killing of all individuals followed the guidelines of the American Fisheries Society (AFS, 2004). All sampled individuals were in the post spawning stage, according to the histological scale employed by Macchi & Christiansen. The swim bladder with its attached sonic muscles was removed; pieces of sonic muscles were frozen by immersion in liquid nitrogen for 60s and stored at -25° C. Sections were cut at 12 µm in a cryostat and then mounted. Slides were stained with haematoxilyn and eosin (H-E). The following histochemical techniques were done: a) for myosin adenosine triphosphatase (m-ATPase) a modified test adapted to fish of the Guth & Samaha (1970) method was used (Devincenti *et al.*, 2009). Sections were pre-incubated at room temperature in a range of pH 4.3–10.6 for various periods of time. A control procedure with sodium glycerophosphate in place of ATP was carried out. b) The activity of the oxidative enzyme succinic dehydrogenase (SDH) to detect mitochondria was demonstrated using the nitroblue tetrazolium technique. The controls were slides treated with sodium malonate as an inhibitor (Defendi & Pearson, 1955). c) Periodic Acid Schiff (PAS) to evidence glycoconjugates with oxidizable vicinal diols and/or glycogen. As a control, the procedure was carried out after treatment of the sections with alpha-amylase for 45 min (McManus, 1948). d) Sudan Black and Red to determine lipids. Control tests were carried out with acetone (Chayen *et al*., 1973).

No less than 100 muscle fibre diameters were measured at x312 magnification directly from sections stained using the m-ATPase and H-E techniques according te Kronnie *et al*., (1983). The mean fibre diameter of the sonic muscle was compared with those of the white fibres of myotomal -posterior region- (Devincenti *et al*., 2000) and pectoral fin -deep zone- muscles (Devincenti *et al*., 2009). Statistical analysis was performed using the Kruskal-Wallis one-way analysis of variance test. Comparisons between pair of samples were made applying the Mann-Whitney U test (Zar, 2010).

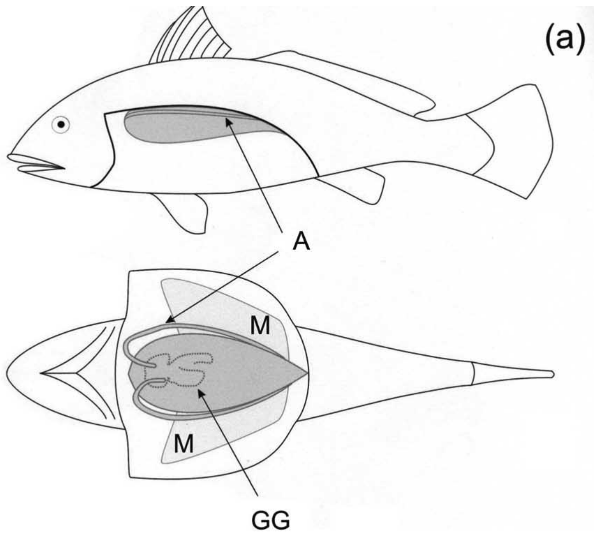
RESULTS

Anatomical description. The sonic organ of *M. furnieri* consists of a swim bladder of the physoclist type and a pair of joined extrinsic muscles located at both sides of the swim bladder which are present in both females and males.

The swim bladder is located along the dorsal wall of the abdominal cavity. The anterior portion is rounded in shape, thinning toward the posterior region. Two tubular caeca extend along the lateral walls of the bladder, bending at the anterior region to the caudal zone. The swim bladder is unicamerate and its gas gland is located in the anterior zone of the bladder's ventral face. The sonic muscles are extrinsic pairs derived from the body musculature. They are placed in the wall of the abdominal cavity on both sides of the swim bladder, having a connection at the posterior zone through a fascia of connective tissue (Fig. 1).

Histology. The sonic muscles were covered by a layer of dense connective tissue where blood vessels and nerves entered; they were divided into fascicles limited by the perimysium. In turn, every muscle fibre was surrounded by the endomysium, where capillaries providing rich blood supply were observed.

The fibres ran parallel to the longitudinal axis of the body, showing homogenous size and distribution. The average fibre diameter was 36.77 ± 6.00 µm, which statistically differed from the mean diameters of the pectoral fin (64.4 ± 26.00 mm) and myotomal (81.52 ± 34.93 µm) white muscle fibres (Fig. 2). Because of the small sample size, genders were not statistically compared. However, they showed similar muscle fiber size.



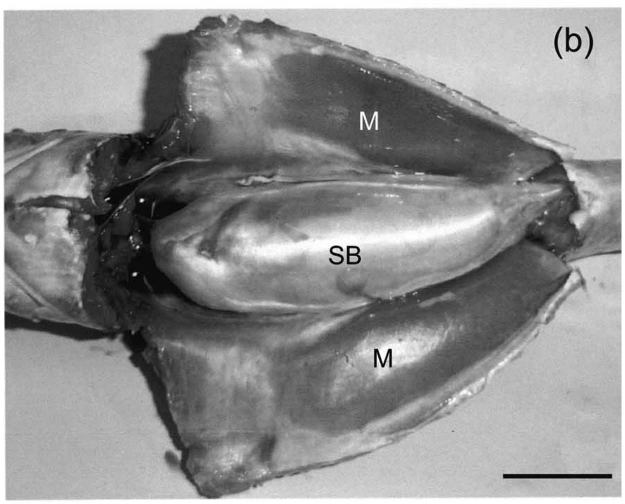


Fig. 1. (a) Scheme of *Micropogonias furnieri* in lateral and ventral positions, (b) Ventral view showing the swim bladder and muscle. A: appendices; GG: gas gland; M: sonic muscle; SB: swim bladder. Scale bar: 6 cm.

The fibres had a sarcoplasmic central zone surrounded by a darker peripheral zone -cylinder zone- that contained the myofibrils. Nuclei were peripheral (Fig. 3a).

Histochemistry. The histochemical techniques here developed revealed a single type of fibre in the sonic muscle of *M. furnieri*. The SDH reaction rendered a negative activity, no lipid deposits were found and the PAS reaction was weak (Fig. 3b). The fibers showed a clear central zone with no m-ATPase activity, surrounded by a peripheral portion, positive to the m-ATPase. The m-ATPase activity at alkaline pre-incubation pHs was moderate, and weak at pH=4.3 (Figs. 3c-d). A summary of the histochemical profile of the sonic muscle of *M. furnieri* is given in Table I. The gonadal stage analyzed showed no histochemical differences between muscle fibres of males and females.

DISCUSSION

The sound organ of *M. furnieri*, just like that of other members of the family Sciaenidae, *Cynoscion regalis* and *Kathala axillaris* (Connaughton *et al*., 1997; Veerappan *et al*., 2009), or members of other families like Carapidae, *Carapus acus* (Parmentier *et al*., 2003), consists of a swim bladder and a pair of extrinsic muscles.

Sound production comes from the vibration of these striated muscles over the wall of the swim bladder. The Atlantic searobin *Prionotus nudigula*, the toad fish *Opsanus tau*, the midshipman *Porrichthys notatus* and the black drum *Pogonias cromis*, all of them have intrinsic muscles completely or partially joined to the wall of the swim bladder (Fine *et al*., 1993; Lindholm & Bass, 1993; Pons, 2006; Locascio *et al*., 2007; Telechea *et al*., 2011); in *M. furnieri* instead, the muscles contact with the bladder, without being straightly joined to it .

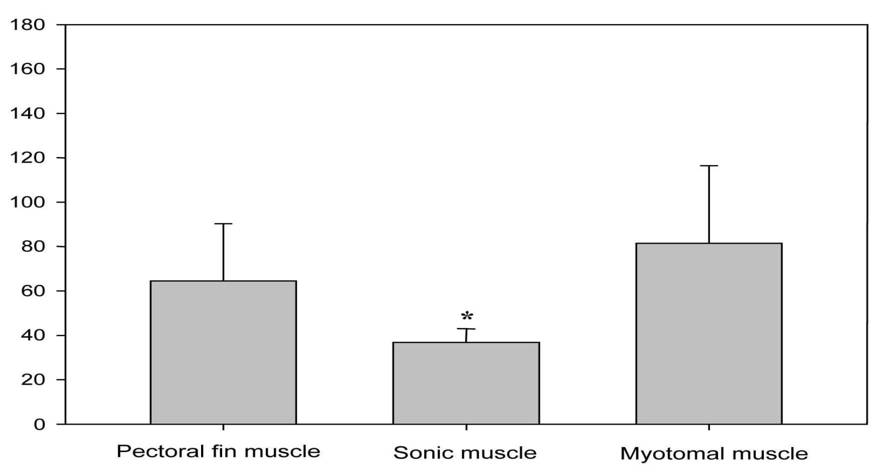


Fig. 2. Mean fibre diameters in pectoral fin, sonic and miotomal muscles of *Micropogonias furnieri*. Values are expressed in µm. Bars denote standard error. * P<0.001 vs. the other muscles.

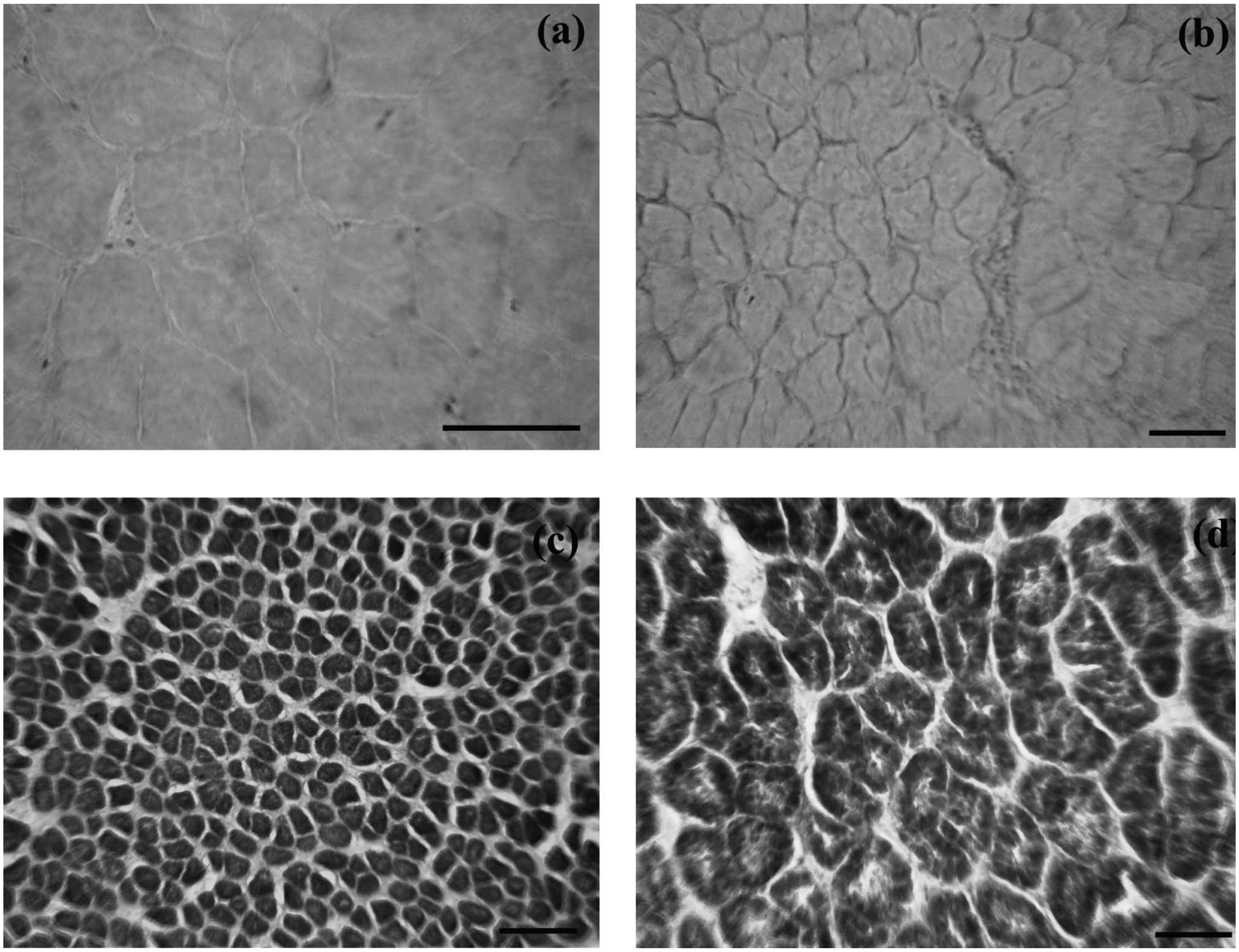


Fig. 3. Transverse section of *Micropogonias furnieri* sonic muscle. (a) H-E, (b) staining with PAS, (c-d) m-ATPase staining after preincubation at pH 10.4. Scale bar: (a, b, d) 40 µm; (c) 120 µm.

Table I. Histochemical staining intensities of the sonic muscle of *Micropogonias furnieri*.

Reaction	Activity
PAS	1
SDH	0
Sudan Black	0
Sudan Red	0
m-ATPase / pH 10.4 5 min.	2
m-ATPase / pH 10.2 5 min.	2
m-ATPase / pH 9.8 5 min.	2
m-ATPase /pH 4.3 1 min.	1

References: 0: negative reaction, 1: weakly positive reaction, 2: positive reaction.

Diameter differences between the sonic muscle and other striated muscles have been also found in *Terapon jarbua* (Chen *et al.*, 1998). In *M. furnieri*, myotomal white fibres and those from the pectoral fin muscles of the deep zone show a mosaic arrangement; fibres of different diameter and different m-ATPase activity at alkaline and acid pHs are present (Devincenti *et al*., 2000; 2009). The sonic muscle fibres, instead, show small and homogeneous diameters, and they have m-ATPase activity at alkaline pHs only. The small size of the fibres would increase the metabolic rate and the elimination of waste products (Fine *et al.,* 1990).

The sonic muscle induces sound production by contracting and relaxing at high speed, thereby forcing the bladder into motion (Ladich & Fine).

The negative reaction of oxidative enzymes, the scarce content of glycogen, the absence of lipid deposits, and the high and stable m-ATPase activity at alkaline pHs, show that the sonic muscle fibres of *M. furnieri* contract rapidly and are fast fatigable. In contrast, in other fishes the sonic muscle is exclusively made up of type IIa fibres, that is glycolytic oxidative (*O. tau*) (Fine & Pennypacker, 1988), or it presents IIa fibres accompanied by a small amount of IIc fibres (*T. jarbua*) (Chen *et al.*).

The fibres of the sonic muscle show a clear central zone with no m-ATPase activity, surrounded by a peripheral portion, positive to the m-ATPase. In *O. tau* the transmission electron microscopy revealed that in the central part or core mitochondria, lipids and glycogen were located, while in the peripheral zone, myofibrils and abundant endoplasmic reticulum were present. This arrangement was interpreted as an adaptation to high contraction speed of the sonic muscle (Fine *et al.*, 1993)

In some species of the family Sciaenidae, such as *C. regalis* and *Leiostomus xanthurus*, the males alone possess a developed sonic muscle, highly specialized in the production of sound associated to courtship (Hill *et al*., 1987). In the weakfish *C. regalis* the androgens produce fiber hypertrophy during the spawning period (Connaughton *et al.*, 1997).

In *M. furnieri* as well as in other sciaenids, the sonic muscle associated to the bladder is found in both sexes (Ueng *et al*., 2007; Tellechea *et al*., 2010; 2011), but males and females sounds have different frequencies. Extrinsic sonic muscles of *M. furnieri* generate disturbance calls produced by both sexes and advertisement or courtship calls produced exclusively by males (Tellechea *et al*., 2010). These two types of sounds were also reported in intrinsic sonic muscles of the black drum *P. cromis* (Locascio *et al*.; Tellechea *et al*., 2011). The sounds of black drum, as in the weakfish, exhibit ontogenetic changes that correlate with the development of the sonic muscle and swimbladder. Sounds change as sonic muscles and swimbladders increase in size (Connaughton *et al.*, 2002; Tellechea *et al*., 2011).

The morphology of the sonic muscle of *M. furnieri* showed no differences between sexes, probably due to the fact that the individuals analyzed were in a post spawning condition. This similarity would indicate a similar sound production in both sexes, related to the "disturbance calls".

This is an original and preliminary research. As a result it will be the starting point for further studies comparing the sonic muscle of males and females at different gonadal stages and all along the ontogenetic development of this species as well as of others related to it.

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RESUMEN: El presente trabajo estudia la morfología del músculo del sonido de *Micropogonias furnieri* por medio de técnicas histoquímicas y lo compara con trabajos previos de la histoquímica del músculo pectoral y miotomal. Con el fin de clasificar las fibras musculares se utilizaron distintas técnicas: succinato deshidrogenasa (SDH) para mitocondrias, ácido periódico Schiff (PAS) para evidenciar glucógeno, Sudan Black y Red para lípidos y miosin adenosina trifosfatasa (m-ATPasa) preincubada a pH alcalinos y ácidos para determinar la velocidad de contracción muscular. Las fibras del músculo del sonido son más pequeñas que las fibras blancas del músculo miotomal y pectoral, presentan un tamaño y distribución homogéneos, y tienen características histoquímicas de fibras blancas: son negativas para SDH y lípidos, débilmente positivas al PAS y a la técnica de m-ATPasa luego de preincubación ácida, y positivas a esta última técnica luego de preincubaciones alcalinas. La morfología del músculo del sonido de *M. furnieri* no mostró diferencias entre sexos, probablemente debido a que los individuos utilizados se encontraban en el estadio de post-desove. Esta similitud estaría relacionada con el tipo de sonido de "disturbio" que es emitido tanto por machos como por hembras durante todo el año.

PALABRAS CLAVE: Músculo del sonido; *Micropogonias furnieri*; Morfología; Histoquímica.

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