Histochemical Characteristics of Sonic Muscle Fibers in Tigerperch, *Terapon jarbua*

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Shueh-Fen Chen, Bao-Quey Huang and Yu-Yi Chien (1998) Histochemical characteristics of sonic muscle fibers in tigerperch, *Terapon jarbua. Zoological Studies* **37**(1): 56-62. Histochemical typing of sonic muscle fibers was investigated in tigerperch (*Terapon jarbua*) by examining the glycogen and lipid contents to determine the energy source, and the activities of the oxidative enzymes SDH, NADH-TR, LDH, and mATPase (with alkaline and acid preincubation) to identify metabolic pattern. The presence of abundant glycogen and little lipid provides the muscle fibers with potential fuel as the energy source and thus supports the designation of sonic muscle as fast oxidative glycolytic fibers. The majority of sonic muscle fibers are type IIa, which suggests that they are metabolically adapted for rapid contraction and fatigue resistance. A minority of sonic muscle fibers are type IIc; these are scattered in the muscle core and show higher responses to oxidative enzymes than do IIa fibers.

Key words: Sound muscle fiber, Energy source, Enzyme activity, Metabolic pattern.

Most teleosts present 2 distinctive types of trunk musculature: 1) slow or red cruising muscle, and 2) fast or white burst muscle (Bone 1978, Love 1980). At higher concentrations of glycogen, red muscles metabolize the glycogen aerobically. White muscles by contrast use glycogen as the primary contents for anaerobic metabolism (Love 1980). Sonic muscles (sound-producing muscles in teleosts) have been commonly classified as red muscle because of their color and because their fibers have a high ratio of sarcoplasm to myofibrils, abundant mitochondria, and small fiber diameters (Schneider 1967). On the contrary, sonic muscles show characteristics of white muscle because of their rapid contraction and because their fibers have an extensive sarcoplasmic reticulum and a well-developed transverse tubular system (Fawcett and Revel 1961, Odense et al. 1978).

Mammalian skeletal muscle fibers, categorized as type I and type II, have different contractile properties. Type I are slow fibers with numerous mitochondria and positive staining responses to various oxidative enzymes. Fast type II have different characteristics of enzyme activities, in particular different reactions after acid preincubation with myosin ATPase, and they are further subdivided into IIa, IIb, and IIc (Dubowitz 1985). Because myosin ATPase activity is closely correlated with the contraction rate (Guth and Samaha 1969, Barnard et al. 1971, Barany 1976) and the redox activities of enzymes are associated with resistance to fatigue, fibers with different enzyme characteristics can reveal different physiological functions and provide the means of identification for typing muscle fibers. Therefore, the adaptive ability in response to different physiological requirements is reflected in the polymorphic types of teleost muscle fibers (Jasra et al. 1991, Meyer-Rochow et al. 1994). Additionally, various fiber types have logically been related to different physiological functions and lifestyle habits (Bone 1978, Higgins 1990, Raso 1991).

Sonic muscles of teleosts are known to have special characteristics which permit extremely fast

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contraction rates, in some cases up to 300 to 400 times/sec (Schneider 1967, Tavolga 1971). Tigerperch (*Terapon jarbua*), "chicken fish" in Taiwan, produce a sound like chicken clucks by contracting their sonic muscles on the swimbladder (Eichelberg 1976, Ueng et al. 1991). The sonic muscle of the oyster toadfish (*Opsanus tau*) is composed uniformly of type IIa as fast-twitch oxidative fibers (Fine and Pennypacker 1988). The sonic muscle of the tigerperch was shown to contain 2 possible types of fibers (IIa and IIc) distinguished by size, responses of mATPase, and their distribution sites in the sonic muscle (Chu et al. 1997).

In order to further describe the histochemical characteristics of IIa and IIc fibers in sonic muscle, properties of the energy source (e.g., storage of glycogen, and lipid content) and activities of the oxidative enzymes (e.g., NADH, SDH, and LDH) were investigated in the present study. NADH provides an index of oxidative capacity, SDH is a key enzyme in Kreb's cycle indicating aerobic metabolism and mitochondrial location, and LDH indicates anaerobic metabolism of pyruvate. In addition, the responses of myosin ATPase after acid or alkaline preincubation as used to distinguish between slow (type I) fibers or fast (type II) fibers (Brooke and Kaiser 1970) were studied in detail to further confirm the fiber types of sonic muscle in tigerperch.

MATERIALS AND METHODS

Tigerperch (*Terapon jarbua*) were obtained from the northeastern coast of Taiwan by hook and line and were held of the aquarium of the Fisheries Science Department, National Taiwan Ocean University. Fishes, 15-20 cm in fork length, were killed by severing the spinal cord. The sonic muscles were exposed on the swim bladder and removed after cutting through the supracleithral bones and subsequently were quickly immersed in isopentane in liquid nitrogen. Serial 10-μm transverse sections were cut in a cryostat (BRIGHT OTF/AS-001) at −20 °C and mounted on slides.

The presence of glycogen was demonstrated by periodic acid Schiff (PAS) (McManus 1948), and that of lipid by oil red O (Lillie and Ashburn 1943). The methods of Nachlas et al. (1957), Nachlas et al. (1958), Pearse (1972) and Chayen et al. (1973) were used to demonstrate succinic dehydrogenase (SDH), NADH-tetrazolium reductase (NADH-TR), and lactate dehydrogenase (LDH) activity, respectively. Sections were also preincubated in alkaline

(pH 10.2 ~ 10.7) or acidic (pH 4.5 ~ 5.0) medium at room temperature, then the calcium method for adenosine triphosphatase (ATPase) activity was applied for differentiating fiber types by the method of Dubowitz and Brooke (1973). All slides were examined and photographed by using an Olympus photomicroscope B201. Cross sections were illustrated by using a camera lucida (Olympus PM-20).

RESULTS

Fiber typing
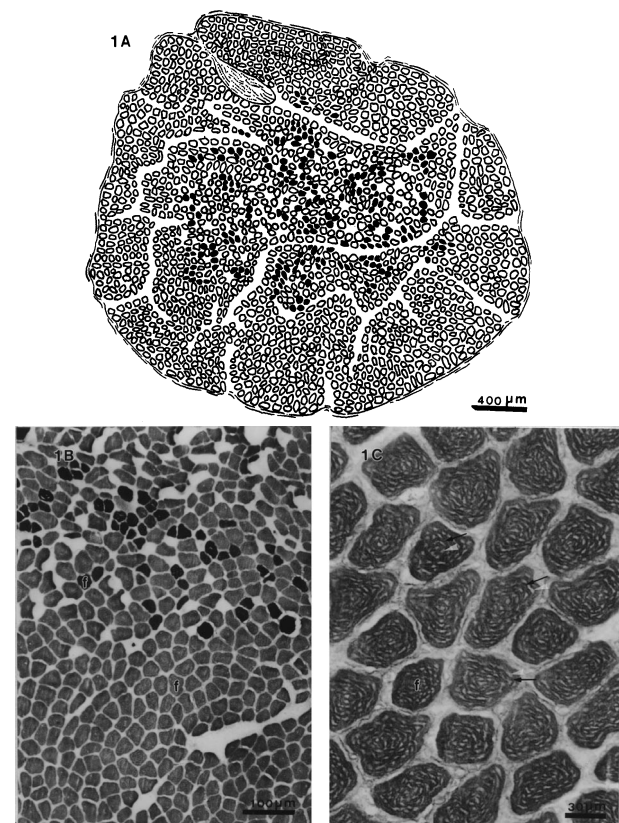
There were 2 distinctively differentiated types

Fig. 1. Cross-sections of sonic muscle of *Terapon jarbua* by camera lucide drawing (A) and after staining (B, C) with alkaline (pH 10.4) mATPase. A few fibers (IIc) with stronger mATPase (dark stain) activity are irregularly scattered in the medullary area of the muscle (A: center area, B: top area), and most fibers (IIa) with weaker mATPase activity (brownish-gray stain) are regularly arranged in the cortical area muscle (B: lower half). Stained with the mATPase method, the major fibers show that the concentric arrangement of myofibrils (black arrow) have been separated by their unstained intermyofibrillar network (white arrow) in the fiber (f).
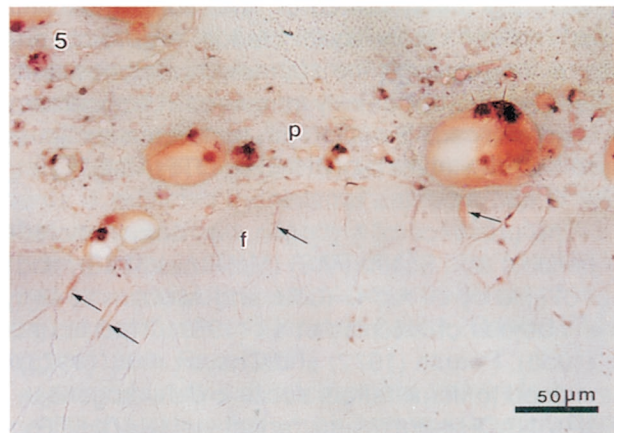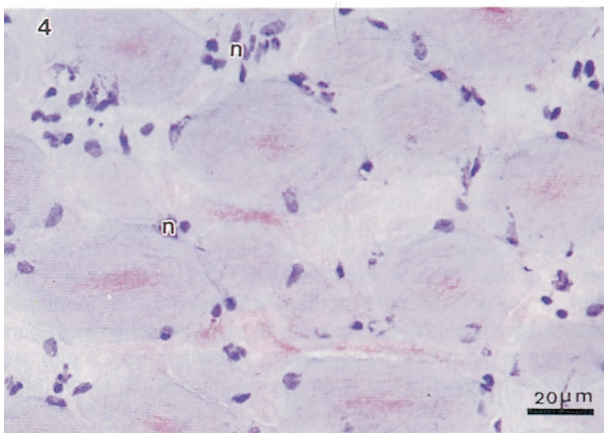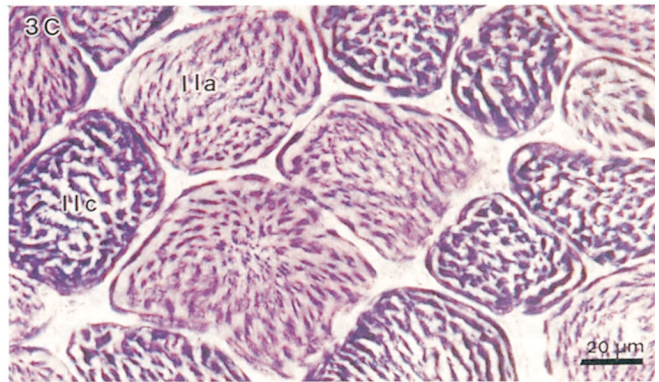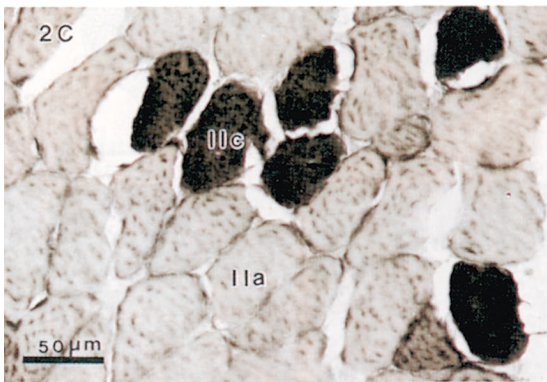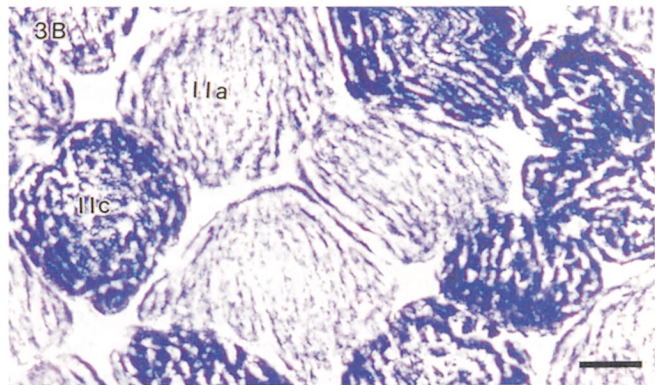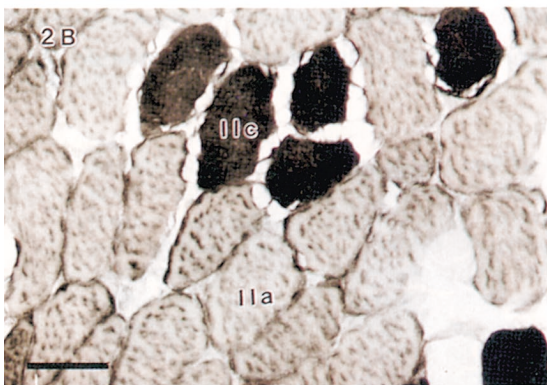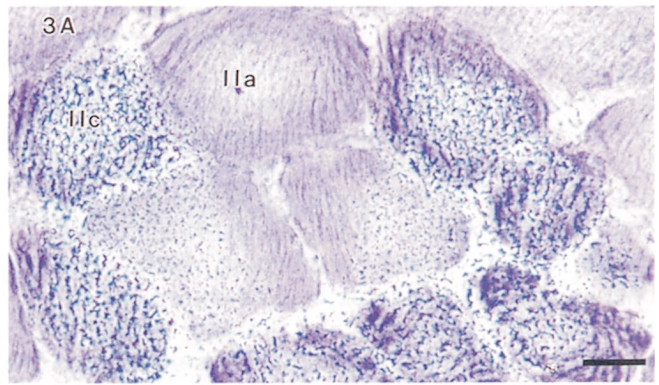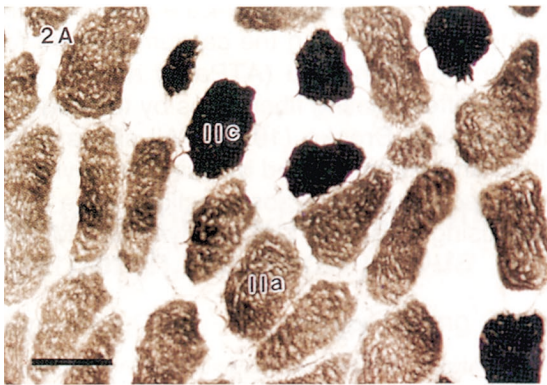
(Caption see next page)

of fibers in sonic muscle of tigerperch seen after alkaline preincubation with mATPase stain (Fig. 1A, B). Most of the sonic muscle fibers with the weaker mATPase activity were densely and regularly arranged in the cortical area of the muscle, but loosely and irregularly arranged in the medullary area (Fig. 1A). We also found that a few fibers with much stronger mATPase activity were sparsely distributed in the medullary area. The myofibrils and the intermyofibrillar network patterns can be more significantly distinguished from fibers of the former type (Fig. 1C). After preincubation in alkaline (pH 10.4) and acidic (pH 4.8 and 5.0) medium (Fig. 2), mATPase activities from serial sections of sonic muscle revealed the distinguishing characteristics between these 2 types. The major fibers were positively stained after alkaline preincubation and less stained after acidic preincubation with the mATPase reaction (Fig. 2), showing the characteristics of IIa fibers. The minor fibers, scattered only in the medullary area of the sonic muscle, were heavily stained after both alkaline and acidic preincubation with the mATPase reaction (Fig. 2), showing the characteristics of IIc fibers.

Histochemistry

In addition to the differential characteristics of mATPase reactions with different pH preincubations, the enzyme activities of SDH, NADH-TR, and LDH between IIa and IIc fibers are distinctively demonstrated in Fig. 3. In serial transverse sections of the sonic muscle, IIa and IIc fibers can be distinguished by their different histochemical characteristics including the different responses to mATPase (Fig. 2). The IIa fibers were not as heavily stained as were IIc fibers by these 3 oxidative enzymes (Fig. 3). The intermyofibrillar network reveals a significant reaction by staining with SDH and presents the characteristic distribution of mitochondria (Fig. 3A). Distinguished according to the

unstained myofibrils, the intermyofibrillar network, comprising the mitochondria and sarcoplasmic reticulum, was distinctively demonstrated by NADH-TR reactions (Fig. 3B). The LDH in the sarcoplasm indicates the various concentrations of the different fiber types (Fig. 3C). Compared to IIa fibers, IIc fibers were more heavily stained by these 3 enzymes, and their intermyofibrillar pattern was also slightly different (Fig. 3).

Energy source

PAS and oil red O stains have been used to identify energy substrate storage patterns in sonic muscle of tigerperch. Stained for glycogen, fine granular appearance of glycogen distribution was localized over the entire cytoplasm of the fibers of both the cortical and medullary areas of the sonic muscle (note the pale pink bands of fibers in Fig. 4). Sonic muscle, by contrast only demonstrates very weak reactions to oil red O stain (Fig. 5). Little lipid is deposited at the surrounding boundaries of each fiber (Fig. 5). A more significant lipid re-

Table 1. Histochemical staining intensities of IIa and IIc fibers in tigerperch (*Terapon jarbua*) sonic muscle

	IIa	IIc
mATPase pre-incubated in pH 10.4 solution	+++	+++
mATPase pre-incubated in pH 5.0 solution	0	+++
mATPase pre-incubated in pH 4.8 solution	0	+++
SDH	++	+++
NADH-TR	++	+++
LDH	++	+++
Glycogen	++	++
Lipid	(+)	(+)

0 = no staining; (+) = staining intensity between 0 and +; ++ = intermediate staining; +++ = heavy staining.

Fig. 2. Fiber types of sonic muscle of *Terapon jarbua* from serial cross-sections by mATPase staining. IIa fibers are heavily stained after alkaline preincubation at pH 10.4 (A), but slightly stained after acid preincubation at pH 5.0 (B) and pH 4.8 (C). In contrast, IIc fibers show very heavy staining after both alkaline and acid preincubation at pH 10.4 (A), pH 5.0 (B), and pH 4.8 (C), respectively.
Fig. 3. SDH (A), NADH-TR (B), and LDH (C) activities in serial cross-sections of sonic muscle of *Terapon jarbua*. IIa fibers show heavy staining in these 3 stains and the intermyofibrillar network (parallel lines) has a clear appearance by SDH stain (A), which indicates mitochondria distribution; NADH-TR (B), and LDH (C) also indicate the intermyofibrillar network pattern (parallel lines). IIc fibers show very heavy staining in these 3 stains. Note that type IIa and IIc fibers have slightly different intermyofibrillar appearances.
Fig. 4. PAS reaction for glycogen. Parallel circular lines with pink color in the center and periphery of fibers indicate the existence of glycogen. Note many nuclei (n) of muscle fibers distributed near the sarcolemma.
Fig. 5. Oil Red O reaction for lipid. Lipid deposits with red color are displayed at the perimysium (p) of sonic muscle (top area), but only a little lipid (arrow) is distributed at boundaries of muscle fiber (f).

sponse can be found only in the perimysium (Fig. 5). However, it is not possible to identify any significant differences between IIa and IIc fibers from either stain reaction in the present study.

Table 1 summarizes the histochemical reactions of the 2 fiber types (IIa and IIc) in the sonic muscle of tigerperch, *Terapon jarbua*. From our results, type IIa fibers have alkali-stable and acid-labile mATPase activities, these fibers are also positively stained with SDH, NADH-TR, and LDH reactions. Type IIc fibers have both alkali- and acid-stable mATPase activities; these fibers are relatively heavily stained with SDH, NADH-TR, and LDH reactions.

DISCUSSION

Electron microscopic examination of sonic muscle fiber of *Terapon jarbua* reveals characteristics of the white fiber type (Eichelberg 1976). On the contrary, the fine structure of sonic muscles in *Terapon jarbua* demonstrates some characteristics of red fibers with small diameter (17-20 μm, compared to 50 μm in its own trunk muscle), with extremely numerous mitochondria and a massively developed sarcoplasmic reticulum (Eichelberg 1976). The classification of fish muscle fibers is generally based upon innervation, contraction speed, and metabolic characteristics rather than simply by color (Johnston 1983). A previous study showed that polyaxonal and multiple innervations are present in the sonic muscle of tigerperch (Chu et al. 1997). The muscle fibers with polyaxonal and multiple innervation were suggested to fire maximally in a synchronized fashion, and not to make graded responses (Fine and Mosca 1989, Bass and Baker 1990). Therefore, it is logical that the sonic muscle of *T. jarbua* should possess characteristics of white fibers to meet the requirement of fast twitching, especially with the IIa type in order to produce sound.

The characteristics of enzyme activities presented in this study suggest that sonic muscle fibers in tigerperch can contract rapidly. High SDH activity provides anatomical evidence of utilization of aerobic metabolic activity via Kreb's cycle (Dubowitz 1985). NADH indicates that there is a metabolic pathway converging into the electron transport system, thus permitting more ATP production for fast contraction in sonic muscle (Fine et al. 1986). Positive activity of LDH indicates that the muscle may go into oxygen debt from the pyruvate shunted into anaerobic metabolism (Dubowitz

1985). The capacity for anaerobic metabolism in sonic muscle of tigerperch in the present study is similar to that in *Opsanus beta* (Walsh et al. 1987). These biochemical properties indicate adaptations for increased speed, fatigue resistance, and contraction excitation in order to benefit fast oxidative glycolysis for the specific function of sound production (Odense et al. 1978, Fine et al. 1986, Fine and Pennypacker 1988).

Applying histochemical techniques in the present study, 2 distinctive fiber types are distinguished in the sonic muscle of tigerperch *T. jarbua*. The major fibers of sonic muscle have high SDH, NADH-TR, and LDH activities and alkali-stable and acid-labile mATPase activities, which are similar to those of type IIa fibers of mammals (Dubowitz 1985) as fast oxidative glycolytic (FOG) (Peter et al. 1972) or as fast fatigue resistant (FR) (Burke et al. 1973) fibers. The less frequent fibers, with very high SDH, NADH-TR, and LDH activities and both alkali-and acid-stable mATPase, are similar to those of type IIc fibers of mammals (Dubowitz 1985). Since the only function of this muscle is sound production, the rapid and synchronized contractions in making sound are consistent with those of type IIa fibers. The IIc fibers might represent a precursor of the other muscle types (type I, IIa, or IIb fibers) which can differentiate, into IIa or IIb fibers (Brooke et al. 1971, Dubowitz 1985).

Cross-sections of sonic fibers show 2 discrete areas with sizes ranging between 681.25 ± 184.27 μm² (*n* = 945) in the major fibers (IIa) and 402.02 ± 178.86 μm² (*n* = 308) in the minor fibers (IIc) (Chu et al. 1997). With the higher oxidative enzyme activities seen in the present study and with smaller diameters than those of major fibers in a previous report (Chu et al. 1997), the minor fibers are more similar to red fibers, although they show different mATPase responses from those characteristic of type I. Since red, or type I, fibers belong to slow twitch fibers type, and sonic muscle fibers are designed to fire maximally in a rapid and synchronized contractions in sound production, the minor fibers in the sonic muscle do not appear to play a crucial role in making sound. In addition, Fine and Pennypacker (1988) found the sonic muscle of the oyster toadfish (*Opsanus tau*) to be made uniformly of type IIa fast-twitch oxidative fibers. Therefore, it is reasonable to suggest that the major fibers (IIa) are the appropriate fiber type for sound production in tigerperch.

The sonic muscle of *T. jarbua* contains high concentrations of glycogen but no fat deposits (Eichelberg 1976). In sonic muscle of toadfish (*O.*

tau), concentrations of glycogen and fat were 2.2 and 6.2 times higher respectively, than those in its own white trunk muscle (Fine et al. 1986). In the present study, sonic fibers of tigerperch had only sparse lipid distribution along peripheral cellular boundaries and high concentrations of glycogen which indicates that the sonic fibers mainly depend on glycogen as the energy source for metabolism. Abundant glycogen also contributes to the important characteristics of fast oxidative glycolytic fibers (Raso 1991). In the present study, glycogen granules distributed in both central and peripheral sarcoplasm may be useful for providing their released energy (Fine et al. 1993). However, the small lipid content in tigerperch sonic muscle may be only for forming the lipoprotein complex which exists around the inside and outside of the cell membrane, and does not function as an appreciable energy source (Meyer-Rochow et al. 1994).

Based on enzyme histochemistry in the present work and morphometric parameters in a previous report (Chu et al. 1997), we conclude that (1) most sonic muscle fibers (type IIa) of tigerperch, as those in toadfish (Fine and Pennypacker 1988), uniquely execute the function in sound production; (2) the rarer fibers, scattered in the core area of sonic muscle with mATPase characteristics of IIc fiber, play an uncertain role in sonic muscle. The fine structures and the exact functions of IIa and IIc fibers in tigerperch sonic muscle will be the subject of future research.

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花身雞魚發音肌纖維之組織化學特性

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　　為研究花身雞魚(*Terapon jarbua*)發音肌之肌纖維(肌細胞)類型、能量來源、代謝型式及組成該肌肉之 SDH，NADH-TR，LDH 和 mATPase 等酵素的活性，本實驗利用 PAS 及 oil red O 探討其肝醣與脂肪之含量，並利用肌纖維對 SDH，NADH-TR 和 LDH 等氧化酵素的反應活性，及肌纖維內肌微纖維(myofibril)之 ATP 酵素活性，以分辨纖維之種類。結果顯示發音肌含有大量的肝醣和少量的脂質作為代謝的能量來源，以配合發音肌之快速氧化醣解作用(fast oxidative glycolytic)。另由連續切片之各種酵素反應活性顯示，發音肌主要由 IIa 型肌纖維所組成，此類纖維具對鹼安定與酸不安定的 mATPase 特性，及大量的氧化酵素，為執行發音之功能；另一類數量較少且只散佈於發音肌中央部位之肌纖維屬於 IIc 型，此類纖維同時具有對鹼與酸安定的 mATPase 特性，及更強的氧化酵素活性，並不直接扮演發音的角色。

關鍵詞： 發音肌纖維，能量來源，酵素活性，代謝型式。

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