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Fibre Composition of Pectoral Fin Muscles in the Murrel, *Channa punctata* (Bloch): A Histophysiological Investigation

C L TALESARA and A J URFI
Muscle Physiology and Histochemistry Unit,
Department of Zoology, University of Delhi, Delhi 110007

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The present study describes the distribution of the three principal fibre types: red (or slow), white (or fast) and pink (or intermediate) in two pectoral fin muscles, the adductor and dilator of *Channa punctata*. Both these muscles are essentially slow as revealed by histochemical staining for SDH and myofibrillar ATPase. Quantitative assays for these enzymes and LDH isozyme have been done by PAGE. In the light of this study the role of pectoral fin muscles in swimming and the scope for future experimental work on fin muscles is briefly discussed.

Key Words: **Pectoral fins, Adductor, Dilator, Myofibrillar-ATPase,** *Channa punctata*

Introduction

Some studies on fin propulsion (Webb 1973a & b) have shed light on their efficiency for generating thrust. It has also been hypothesized that the abduction and adduction strokes of the pectoral fins generate lift in a fashion which is quite similar to the wing beat cycle of birds (Gray 1968).

Many recent studies on the fibre composition of fish muscles have centered around the myotomal (Johnston et al. 1972, 1974, Mosse & Hudson 1977, Bone 1978, Talesara & Shashi Kiran 1983), caudal (Nag 1972, Shashi Kiran & Talesara 1984) and branchial muscles (Hughes & Johnston 1979, Munshi et al. 1975). These studies have confirmed that the distribution of the three principal fibre types red, white and pink or intermediate varies between different fish muscles and most commonly a distinct zonal seggregation is encountered in the lateral myotomal mass. Similar studies on fin muscles had hitherto been neglected and only few accounts on the fibre composition of pectoral fin muscles are available (Johnston & Goldspink 1973, Walesby & Johnston 1980). A more recent study by Johnston and Harrison (1985) has discussed the contractile and metabolic characteristics of pectoral muscle fibres from antarctic fish.

The present histochemical and biochemical study aims at elucidating muscle fibre types for two pectoral fin muscles—Adductor and Dilator, in the common Indian murrel *Channa punctata*. This fish employs it's fairly large, paddle shaped pectoral fins for slow movements and possibly for balancing. The large myotomal mass is active at the time of cruising or burst activity only.

Materials and Methods

Fishes were purchased locally and maintained in glass aquaria at 25°C on a diet of chopped rat-liver. The water in the tanks was replaced regularly and proper sanitation ensured.

Histochemical Method

15–20 cm long fishes were stunned by a blow on the head and killed by decapitation. On the posterior side the adductor and dilator muscles were carefully dissected out and weighed. Large muscles were cut to a suitable size, mounted on pre-cooled cryostat chucks and quickly frozen by immersion in liquid nitrogen. The blocks were placed in the cryostat (IEC-Model CTD) at −25°C. Frozen sections of 10 μm thickness were cut and used for histochemical studies.

Myofibrillar-ATPase (m-ATPase) and Succinic Dehydrogenase SDH

Air dried sections (60–180 min) were stained using the routine m-ATPase method of Padykula and Herman (1955) after pre-incubating sections in acid or alkaline medium as described by Guth and Samaha (1970). In each case the incubation was carried out at 37°C. *p*H-9.4 for about 15 minutes. The pre-incubation period varied from a dip of 5 sec to 6 minutes. For SDH fresh frozen sections were incubated for 15 minutes at 37°C in the medium described by Nachlas et al. (1957) using Nitro-BT as an electron acceptor.

Biochemical Methods

Myofibrils were prepared according to the method described by Perry and Grey (1956). The reaction for m-ATPase was carried out in a water bath maintained at 37°C, *p*H 7.5 and the other details of the procedure were the same as reported earlier (Talesara & Goldspink 1978, Talesara & Shashi 1983). Ca^{++}-ATPase activity was expressed as μ moles Pi liberated/mg protein/min. The assay procedure for SDH was the same as given by Nachlas et al. (1960). Protein concentrations were determined by the method of Gornall et al. (1949).

Electrophoretic Methods

LDH isozymes were separated by 7% polyacrylamide gel electrophoresis (PAGE) according to Davis (1964) after preparation of homogenized samples in chilled saline EDTA buffer (*p*H 8.0). Other procedures were the same as described earlier (Talesara & Shashi Kiran 1984).

Results and Discussion

Depending upon the locomotory habits of the fish, numerous variations of a basic ground plan are encountered among the pectoral fin muscles of different species (Howell 1933). In the murrel the adductor muscle occurs somewhat as triangular mass and occupies a large space on the posterior side of the pectoral girdle (figure 1). As indicated in table 1, this is the heaviest muscle in the entire pectoral fin complex. It has a proximal insertion onto the pectoral girdle and radiates distally into a fan-shape with multiple insertions near the bases of the fin rays. The other, the posterior dilator muscle runs approximately at right angles to the adductor muscle. It may function in adjusting the leading edge of the pectoral fin. On the anterior side of the girdle the abductor muscle occurs as a thin triangular sheet.

On the basis of colour the adductor muscle consists of two regions (figure 2) separated by a layer of connective tissue. They are a somewhat eresentric red region (R) adjoining the clavicle and the other is the white region (W) which contains numerous small triangular fibres (figure 3).

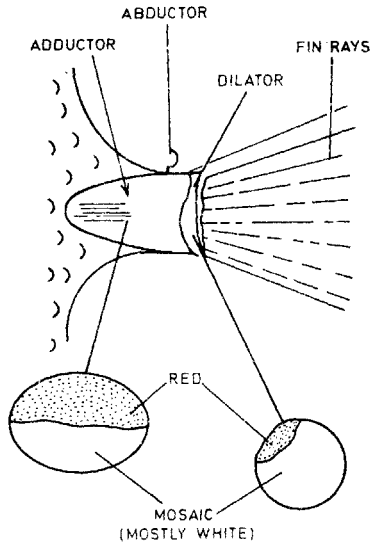

Figure 1 Diagram showing the arrangement of different pectoral fin muscles of *Channa punctata*. The distribution of principal fibre types in adductor and dilator muscles are shown in cross section

The red region (R) which is predominantly composed of small diameter red or slow fibres, show intense reaction for SDH, and also has numerous medium diameter 'pink' or intermediate fibres which exhibit moderate stain. On the basis of histochemical stain for m-ATPase (alkaline preincubation *p*H 10.4—figure 4) and SDH (figure 5) both these fibre types are differentiated and together they appear to be arranged in groups or clusters which resemble fasicula in a mammalian muscle. Some other studies on pectoral fin muscles have shown the presence of slow fibres in the adductor (Johnston & Goldspink 1973, Nishihara 1967).

The white region (W) predominantly consists of large diameter white or fast fibres showing intense stain for m-ATPase (figure 6) and very little for SDH (figure 7). Interspersed among these are numerous smaller diameter fibres which show some stain for lipids (figure 3) and SDH (figure 7).

Histochemical staining for SDH (figure 8) and m-ATPase (figure 9) after alkaline preincubation (*p*H 10.4) demonstrates the presence of all three fibre types viz. red, pink and white in the posterior

Table 1 *Average weights* for pectoral fin muscles (in mg) of* Channa punctata *belonging to two different length groups (cm)*

Length group	Adductor	Dilator	Abductor
14.5 – 17.5	75.75 ± 11.087	32.0 ± 4.281	24.0 ± 5.01
21.5 – 24.5	140.00 ± 18.587	55.26 ± 15.896	27.5 ± 1.52

***Values are Mean ± SD. Average are for 7-8 animals in each length group.**

dilator muscle also, which is much smaller than the adductor and lies perpendicular to it (figure 1). These fibres in fish muscle are considered analogous to the slow oxidative (SO), fast oxidative glycolytic (FOG) and fast glycolytic (FG) fibres, respectively of mammalian muscle (Close 1972).

Barany (1967) has shown for a wide variety of vertebrate and invertebrate muscles that there is a good correlation between the speed of muscle contraction and the m-ATPase activity. Biochemical results in the present study indicate that the pectoral adductor is a slow muscle, in that its m-ATPase activity is almost similar to myotomal red muscle and is about 1/3rd of the myotomal white muscle. Correspondingly, its oxidative capacity, as revealed by SDH assay, is many-fold higher than white and quite similar to red myotomal muscle (table 2). Interestingly, in the muscles of an Antarctic teleost, *Notothenia rossii*, a similar pattern of enzyme activity has been observed for m-ATPase. However, in this case the oxidative capacity of the adductor is much higher than either red or white myotomal muscles (Walesby & Johnston 1980). This may be attributable to the fact that the fish, *N. rossi* is truly labriform and so the red muscle in the myotome is not so well developed. This, however, is not the case in *Channa punctata*.

Our previous studies on the isozymes of lactate dehydrogenase (LDH), involved in the final step of anaerobic glycolysis, have dealt with their distribution pattern in different muscles of freshwater teleosts (Talesara & Shashi Kiran 1984). Though LDH isozymes in fish tissues are complex and not homologous to mammalian isozymes a possible generalization from previous studies (Markert 1965) is that red muscle isozymes resemble heart in having more **H** type subunits. White muscle has more **M** type subunits in contrast. Due to the absence of any prior studies on fin muscles, we have described the LDH-isozyme patterns also for the adductor and dilator in relation to other muscles of *Channa punctata* (figure 10).

The coordination and contraction pattern of the pectoral fin muscles is a matter of conjecture at present. However, their high aerobic capacity and low ATPase (or slow speed of contraction) is hardly surprising. As laboratory observations indicate they are in constant use and employed by the fish to produce slow movements. The high degree of manoeuvrability of the leading edge of the fin is perhaps attributable to the dilator muscle. Undoubtedly, investigations on its innervation pattern and properties of proprioception (if any) will shed further light on its role. Above all, studies on the light chains (low molecular weight proteins of m-ATPase) will be a useful clue towards understanding their true nature.

Numerous investigations have been carried on the behaviour of vertebrate muscle fibres to variable loads, disuse and denervation. For fish muscle this aspect has received scant attention. We feel, that the pectoral fin muscles because of their unique structure and position offer a good material to study this aspect in fish by employing simple methods such as fin clipping or fin amputation. This work is in progress at present in our laboratory. Indeed, in the Sockeye Salmon, Webb (1973)

Table 2 *m-ATPase and SDH activity* in the pectoral adductor and myotomal muscles of* Channa punctata

Particulars	Red	White	Adductor
m-ATPase	0.3095 ± 0.0588	0.8656 ± 0.1719	0.3110 ± 0.0121
SDH	1.096 ± 0.008	0.2820 ± 0.0527	1.095 ± 0.0860

***Values are Mean ± SD.**
m-ATPase: μ moles Pi/mg protein/min;
SDH: μg formazan/mg protein/min.

has explained the effects of partial caudal fin amputation in terms of redistribution of thrust related hydrodynamic loading over the remaining portion of the tail and body. It will be interesting to see whether fin amputation can significantly alter the work-load so as to bring about histochemically or biochemically demonstrable changes in the adductor muscle.

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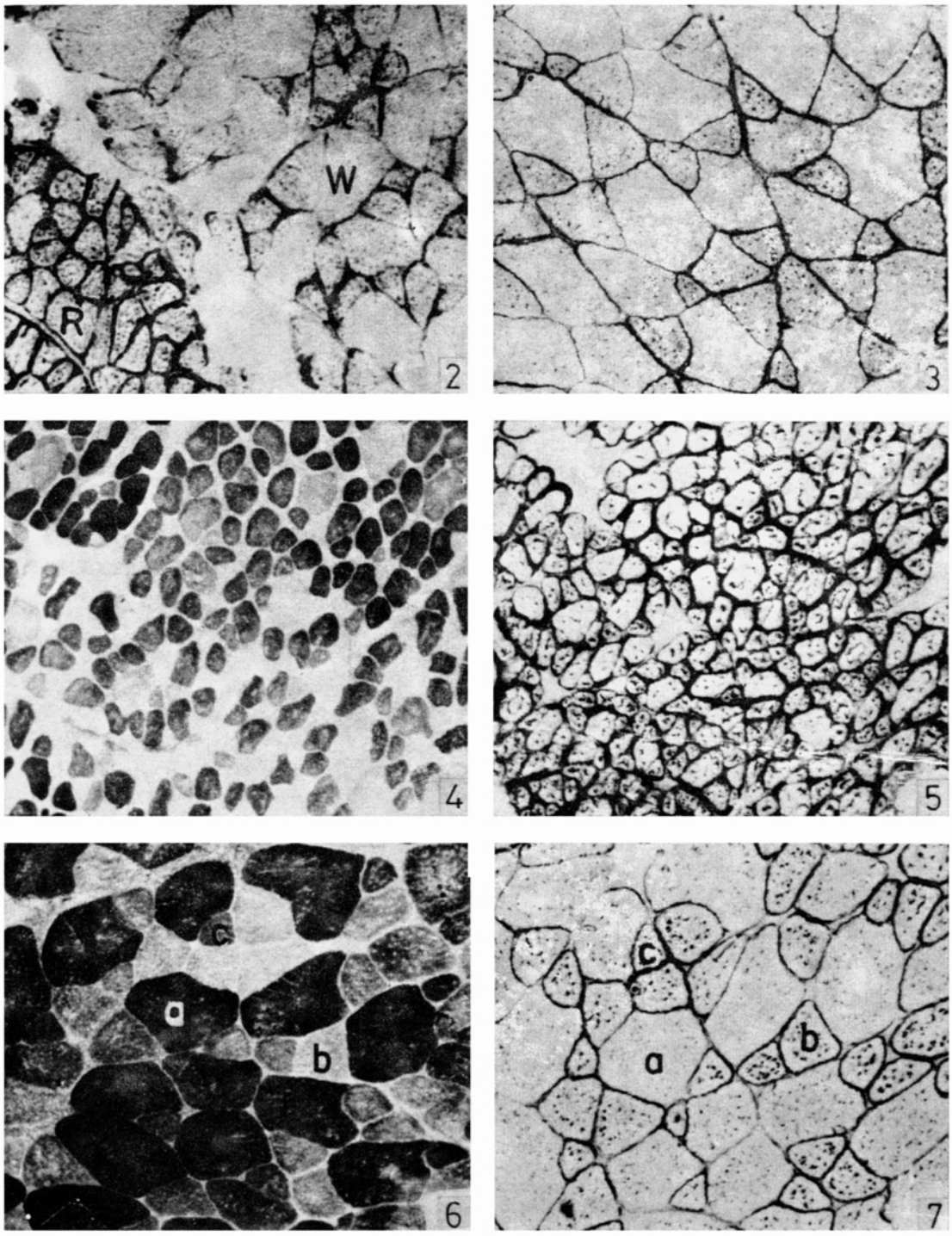
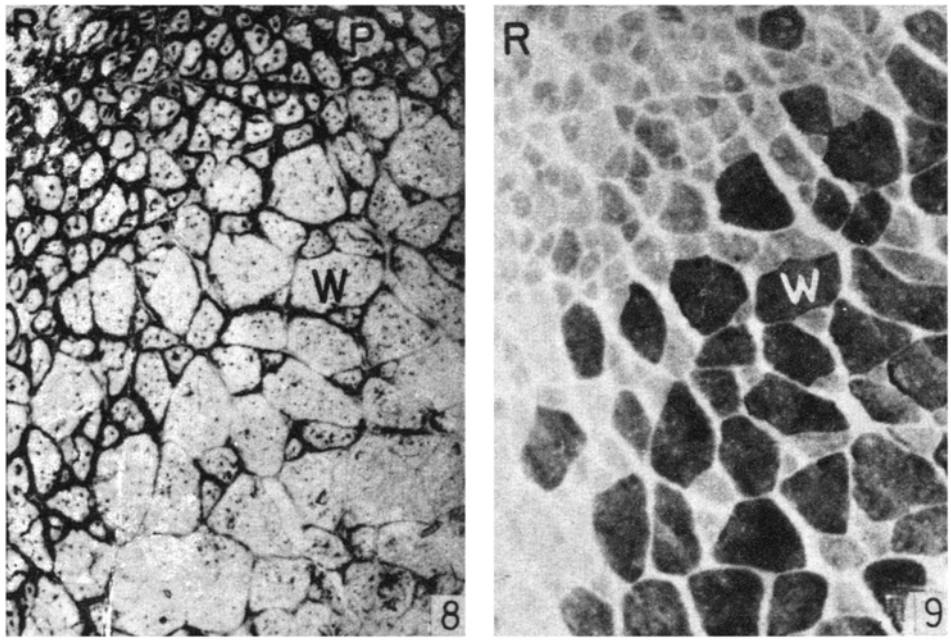


Figures 2–7 Serial transverse sections of adductor muscle of *C. punctata* showing red, pink and white fibres (× 120). **2**, Red (R) and White (W) regions, stained for SDH; **3**, White region stained for lipids; **4–5**; Red region; **6–7** White region; **4 & 6**, stained for m-ATPase after alkaline preincubation (*p*H 10.4); **5 & 7** stained for SDH. Note the corresponding fibres marked as a, b & c in **6 & 7**



Figures 8–9 Serial transverse sections of dilator muscle of *C. punctata* showing Red (R), White (W) and Pink (P) fibres (× 120); **8**, stained for SDH; **9** Stained for m-ATPase after alkaline preincubation (*p*H 10.4)

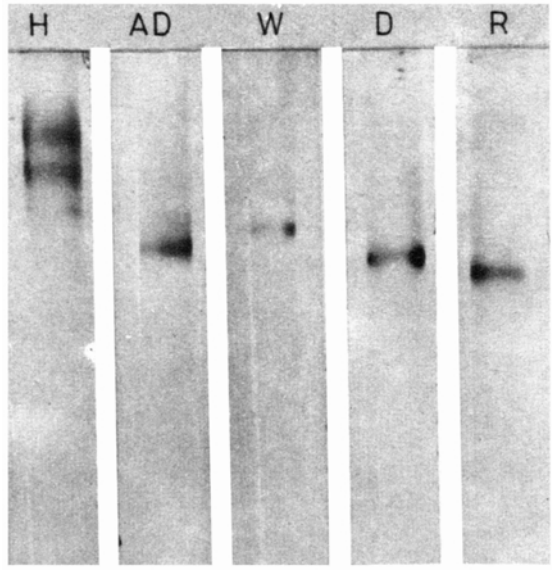


Figure 10 Polyacrylamide gels showing LDH electrophoretic isozyme distribution pattern in myotoma and pectoral fin muscles of *C. punctata* (*H*, Heart; *AD*, Adductor; *W*, White; *D*, Dilator; *R*, Red)