

## A comparative study on certain biochemical aspects of red and white myotomal muscles of the black skipjack tuna, *Euthynnus affinis* Cantor

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**Abstract.** The biochemical assay of certain metabolites of the red and white myotomal muscles of the tuna, *Euthynnus affinis* Cantor has been carried out. The metabolites exhibited a marked variation in their distribution pattern in red and white muscles. The narrow red fibres are characterised by higher levels of lipid, glycogen, myoglobin and SDH while the broader white fibres had lesser amount of the above metabolites. The distribution of metabolites—the myoglobin and SDH, revealed a gradient from the superficial towards the inner layers of the red myotomal muscle in both the pectoral and middle regions. The physiological relevance of these biochemical variations in diverse regions of the red and white muscle is discussed.

**Keywords.** Skipjack tuna ; red and white muscles ; protein ; fuel reserves ; myoglobin ; SDH, *Euthynnus affinis*.

### 1. Introduction

Tuna are actively swimming, commercially important teleosts, exhibiting unique adaptation for maintaining a higher body temperature than the surrounding ambient medium. Similar to certain other teleosts, tuna also possess two types of myotomal muscles—the red and white, with the red muscle lying near the spine and constituting about 5 to 20% of the total body weight (Modigh and Tota 1975). Morphological and biochemical investigations on red and white muscles of fishes have elaborated the functional differences between them (Love 1970). Generally the red fibres are adapted for long-term cruising movements, utilizing lipid as the main source of energy and the white fibres for short-term activity metabolising glycogen as the chief fuel (George 1962 ; Black *et al* 1962 ; Bilinsky 1963 ; Bone 1966 ; Love 1970). However, relatively very little information is available regarding the physiology of these muscles and their nutritional significance in the black skipjack tuna, *Euthynnus affinis*.

Accordingly a study has been undertaken to elaborate the comparative biochemical aspects of these red and white muscles in *Euthynnus affinis* Cantor.

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## 2. Materials and methods

Investigations were carried out on tuna weighing 2 to 3 kg having a range of 35 to 55 cm total body length. The fishes were collected from boats immediately on landing at the Shankumugham Beach at Trivandrum and were transported immediately in refrigerated containers to the laboratory. The muscle samples were excised from the superficial, middle and inner most layers from three different regions of the body, viz, the pectoral, middle and the caudal regions (single sample) for biochemical assay by employing standard analytical techniques. The following methods were employed for the estimation of lipid, glycogen, total protein, myoglobin and SDH.

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|---------------|---|
| (a) Lipid     | Nayeemunisa and Rao (1972)  |
| (b) Glycogen  | Anthrone Reagent technique of Seifter <i>et al</i> (1950)                 |
| (c) Protein   | Wong's microkjeldhal method (1923)  |
| (d) Myoglobin | Tappan and Raynaferjee (1957)   |
| (e) SDH       | Kun and Abood (1949) using the tetrazolium salt as the electron acceptor. |

The optical density of the aliquots obtained was measured in a photoelectric colorimeter (Bausch and Lomb, Spectronic 20).

## 3. Results

The data on biochemical aspects of these muscles are shown in table 1. Regarding moisture content, the pectoral and middle regions of the red muscle showed only a very slight variation between the superficial layer and the inner layer (Pectoral PS-69.68% ; PM-70.71% and PI-70.97% and middle MS-70.09% ; MM-69.88% and MI-69.60%) with the caudal region exhibiting a moisture level of 69.84%. The white muscle exhibited relatively higher percentage of moisture 72.57%.

Comparatively, the protein levels of the red muscle did not reveal any variation with the middle layer of the pectoral region having the maximum amount of protein (20.08%). The inner layer of both pectoral and middle regions exhibited slightly lower values (19.23% and 19.65%). The caudal region had 19.91%. The white muscle exhibited a relatively higher protein level (22.48%).

The maximal amount of lipid has been recorded from the middle layers of pectoral and inner layer of middle regions (17.02% and 14.98%). The superficial layers of these regions showed slightly lower values of lipid (15.07% and 13.26%). Interestingly enough both these regions exhibited a gradient in distribution of lipid with the maximum being at the inner and minimum in the superficial. The caudal region had a lower level (12.42%) of lipid. Regarding the white muscle the lipid concentration was very much lower than that of the red muscle (6.96%).

The middle layers of pectoral and middle regions exhibited maximal quanta of glycogen (PM-300.95 and MM-365.85  $\mu\text{g}/100\text{ mg}$ ) with a minimal amount at the inner layer (PI-230.99 and MI-262.63  $\mu\text{g}/100\text{ mg}$ ). The superficial layers showed 233.38 and 284.49  $\mu\text{g}/100\text{ mg}$  of glycogen in the pectoral and middle regions respectively. A higher amount of glycogen (323.61  $\mu\text{g}/100\text{ mg}$ ) was

Table 1. Biochemical composition of red and white muscle of *Euthynnus affinis* Cantor.

Body regions	Moisture (% gm wet wt.)	Protein (% gm wet wt.)	Lipid (% dry wt/gm)	Uricogen ( $\mu$ g/100 mg wet tissue)	Myoglobin (mg/gm wet tissue)	SDH ( $\mu$ gm formazan min/g)
Pectoral superficial	69.68 $\pm$ (0.32)	19.79 $\pm$ (0.40)	15.07 $\pm$ (0.37)	233.3557 $\pm$ (30.87)	8.6293 $\pm$ (0.30)	42.85 $\pm$ (4.15)
Pectoral middle	70.71 $\pm$ (0.12)	20.08 $\pm$ (0.29)	17.02 $\pm$ (0.56)	300.9526 $\pm$ (30.65)	12.0627 $\pm$ (0.62)	57.46 $\pm$ (6.18)
Pectoral inner	70.97 $\pm$ (0.27)	19.23 $\pm$ (0.76)	16.21 $\pm$ (0.52)	230.9875 $\pm$ (32.25)	13.3008 $\pm$ (1.24)	59.19 $\pm$ (5.65)
Middle superficial	70.09 $\pm$ (0.24)	19.93 $\pm$ (0.42)	13.26 $\pm$ (0.59)	284.9942 $\pm$ (36.54)	8.1042 $\pm$ (1.14)	45.67 $\pm$ (10.54)
Middle middle	69.88 $\pm$ (0.27)	19.50 $\pm$ (0.32)	14.07 $\pm$ (0.68)	365.8492 $\pm$ (41.07)	14.5783 $\pm$ (1.21)	62.49 $\pm$ (8.11)
Middle inner	69.60 $\pm$ (0.27)	19.65 $\pm$ (0.28)	14.98 $\pm$ (0.26)	262.6330 $\pm$ (37.30)	15.8272 $\pm$ (1.33)	65.68 $\pm$ (10.59)
Caudal red	69.84 $\pm$ (0.17)	19.91 $\pm$ (0.23)	12.92 $\pm$ (0.49)	323.6126 $\pm$ (40.17)	13.4902 $\pm$ (1.06)	68.41 $\pm$ (9.38)
White muscle	72.54 $\pm$ (0.27)	22.14 $\pm$ (0.49)	6.96 $\pm$ (1.29)	60.1494 $\pm$ (28.85)	2.4635 $\pm$ (0.16)	27.87 $\pm$ (3.68)

The number of fish examined in each case was 10.  
Mean value of observation is given with standard error in brackets.

discernible at the caudal region. As for the white muscle the glycogen concentration was significantly very much lower ( $60.15 \mu\text{g}/100 \text{ mg}$ ).

The myoglobin levels of both pectoral and middle regions exhibited a marked gradient from the superficial to inner layers (PS— $8.6293$ ; PM— $12.0627$ ; PI— $13.3008$  and MS— $8.1042$ ; MM— $14.5783$  and MI— $15.8273 \text{ mg/g}$  wet tissue. The caudal region had  $13.4902 \text{ mg/gm}$  of myoglobin. Relatively, the white muscle revealed only a very much lower value for myoglobin ( $2.4635 \text{ mg/g}$ ).

The concentration of SDH at the pectoral and middle regions exhibited an increasing gradient from superficial towards the inner layers—viz., (Pectoral; PS— $42.85$ ; PM— $57.46$ ; and PI— $59.19$  and middle; MS— $45.67$ ; MM— $62.49$  and MI— $65.68$ ). The SDH level in the caudal region was relatively higher ( $68.41$ ) while the concentration in the white muscle was very much lower ( $27.87 \mu\text{gm—formazan/min/g}$ ).

#### 4. Discussion

The moisture concentration revealed only a very narrow range of variation in the red muscle samples from different regions of the tuna fish. Nevertheless a comparatively higher percentage has been noted in the white muscle. Alexander (1955), Love (1970) and Chinnamma (1975) had reported a higher value of moisture content in white muscles of fishes.

Studies on the protein content of *Euthynnus affinis* did not reveal any significant variation in the red muscle samples. However, protein level was comparatively higher in the white muscle. This is similar to those of the avian muscles such as pigeon pectoralis muscle as reported by Pishawikar (1961). It has been suggested that the higher total protein content in the white muscle is due to structural proteins, such as actin and myosin and the higher water soluble protein in the red fibers is due to their higher enzyme concentration (George and Berger in *Avian myology* 1966). Further, lower levels of total protein had been reported in the dark muscles of *Sardinia melanosticta* (Fujikawa and Naganuma 1936); *Scomber scombrus* and *Thunnus thynnus* (Braekkan 1959) which are also in conformity with the values observed in the dark muscles of *Euthynnus affinis*.

Regarding fuel reserves, data indicate that the red muscle fibres have a higher fuel reserve with a preferential dependence on lipid. Lipid constitutes an important source of fuel reserve for muscle contraction; the metabolism of which yield sufficient ATP (West *et al* 1956). In tuna, the relatively higher level of lipid in the pectoral region may be due to the continuous activity of the pectoral fins. Further, it is also possible that the red muscle fibres are capable of providing the requisite amount of energy for the slow and sustained contraction for the long term swimming activity of the fish by the aerobic oxidation of the lipids. The relatively lower level of lipid in the white muscle may be due to their non-involvement in slow and sustained activity and are mainly meant for fast spurts of movement using mainly glycogen as the fuel. The breakdown of lipid is most evident in those fish which migrate without feeding (Bilinisky 1963). It has been reported that in the trout (*Salmo gairdnerii*) the ability of dark muscles to oxidise fatty acid was much greater than that of the ordinary muscles by the presence of an enzyme system in the red muscle (Bilinisky 1963). The observations of Drummond

and Black (1960) had revealed that fat metabolism provides the energy for sustained swimming in the up stream migration of salmonids. Further, studies of Braekkan (1959) in *Clupea harengus*; *Gadus virens*, *Salmo salar*; Alexander (1955) in *Scatophagus argus* and *Labeo rohita*; George (1962) in *Rastrelliger kanagurta*; Zama (1963) in *Thunnus orientalis* etc., had revealed a much higher lipid concentration in the red muscles of these fish.

Glycogen is one of the major fuel reserves of the muscle. Studies on *Euthynnus affinis* have revealed a higher concentration of glycogen in the red muscle almost ranging over two to three times than that of white muscle. In fishes usually the white muscle produces much of the energy for sudden bursts of activity by anaerobic metabolism (Rayner and Keenan 1967). Apart from this, certain functions have been attributed to the red muscles in fish myotome. Among these, Arloing and Lavocat (1875) have suggested that the two types of fibres—the red and white were active during different phases of swimming. George (1962) had reported in the teleost, *Rastrelliger kanagurta*, the red muscle was adapted for continuous and slow contractions while the white fibres effecting quick and fast contraction. Further, the observations of George (1962); George and Bokdawala (1964); Bone (1966); Love (1970) corroborate the view that the red muscle facilitates continuous muscular activity of the animal.

Comparatively a lower concentration of glycogen was recorded in the tuna white muscle. Bokdawala and George (1967) had suggested that the probable depletion of glycogen in the white muscle may be due to the fact that it might have been used up since the white muscle fibres are involved in quick and sudden movement during capture by utilizing the energy derived from the breakdown of glycogen. Studies by Driedzic and Hochachka (1976) in carp muscle had revealed an increase in glycolytic intermediates during activity. Thus in tuna, *Euthynnus affinis*, the comparatively higher levels of lipid and glycogen in the red muscle indicates the continuous and higher rate of utilization of these fuels for the active swimming habits of this teleost.

The characteristic red colour of the tuna red muscle is due to the preponderance of myoglobin. It plays a salient role in the transport and storage of oxygen in the muscle (Lawrie 1952) and has the capacity for rapid oxygenation and deoxygenation. Thus in tuna, the higher myoglobin levels in the red muscle facilitate a better diffusion of oxygen into the red muscle and function as a store house of oxygen for the aerobic oxidation. Further, in red muscle the main energy source is lipid and it can be metabolised aerobically which warrants a sufficient supply of oxygen. Observations of Modigh and Tota (1975) in *Thunnus thynnus* revealed that mitochondria from deep red muscle consume more than thrice as much oxygen as those from white muscle when the complete electron transport chain is in operation. Moreover, in *Euthynnus affinis*, the higher level of myoglobin in the inner layers of pectoral and middle red muscle regions, wherein the arteries and veins are highly concentrated may possibly have specific role in the production and maintenance of slightly higher body temperature together with the "rete mirabili", which plays a prominent role in these parts of the muscle (Carey 1973).

The data obtained on SDH (succinic dehydrogenase), the prime mover of oxidation in the metabolic process going on in a muscle, indicate that its levels are much higher in the red muscle with an increasing gradient from the superficial to

the inner layers in both pectoral as well as the middle regions. However, the white muscle exhibited a significantly lower SDH level. It is well-known that the level of SDH in different layers of the muscle can be correlated to their oxidative capacity. Further, it provides an indication of the mitochondrial intensity. Hence the higher levels of SDH in the red muscle fibres of the tuna fish reflect its higher oxidative capacity. Similar data have been reported by Talesara and Narang (1979) in mammalian and avian muscles. In fact the relatively higher metabolic demands of red muscle warrant a higher SDH concentration in correlation with the increased myoglobin content.

In consensus, the significant variation discernible in the biochemical parameters, especially fuel reserves, myoglobin and SDH in red muscles are in accord with the specific functional requirements of these red muscles viz., the sustained muscular activity, production of increased metabolic energy for maintaining a higher body temperature than the ambient medium.

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#### References

- Alexander K M 1955 A comparison of the gross chemical composition of red and white muscles in the two fishes *Scatophagus argus* and *Labeo rohita*; *J. Anim. Morphol. Physiol.* 1 58-61
- Arloing S and Lavocat A 1875 Recherches Sur l'Anatomie et la physiologie des muscles stress pales et fonces; *Mém. Acad. Sci. Inscript. Toulouse T. 7* 177-197
- Bilinsky E 1963 Utilization of lipid by fish. 1. Fatty acid oxidation by tissue slices from dark and white muscles of Rainbow trout (*Saimo gairdneri*); *Can. J. Biochem. Physiol.* 41 107-112
- Black E C, Connor A R, Lam K C and Chia W G 1962 Changes in glycogen, pyruvate and lactate in rainbow trout (*Saimo gairdneri*) during and following muscular activity; *J. Fish. Res. Board Can.* 19 409-436
- Bokdawala F D and George J C 1967 A quantitative study of fat, glycogen, lipase and succinic dehydrogenase in fish muscle; *J. Anim. Morphol. Physiol.* 14 223-230
- Bone Q 1966 On the function of the two types of myotomal muscle fibre in elasmobranch fish; *J. Mor. Biol. Ass. U.K.* 46 321-349
- Braekkan O R 1956 Function of red muscle in fish; *Nature (London)* 178 747-748
- Braekkan O R 1959 A comparative study of vitamin in the trunk muscles of fishes; *Fisk. Dir. Skr. Ser. Teknol. Und. Kol.* 3 42
- Carey F G 1973 Fishes with warm bodies; *Sci. Am.* 228 36-50
- Chinnamma George 1975 Biochemical differences between the red and white meat of tuna (*Katsuwonus pelamis*) and changes in quality during freezing and storage; *Fish. Technol.* 12 70-74
- Driedzic W R and Hochachka P W 1976 Control of energy metabolism in carp white muscle; *Am. J. Physiol.* 230 579-582
- Drummond G I and Black E C 1960 Comparative physiology fuel of muscle contraction; *Annu. Rev. Physiol.* 22 169-190
- Fujikawa K and Naganuma H 1936 Chemical composition of sardine, *Sardinia melanosticta* (C and V) from Tyosen. 1. Comparative study on dark muscle and white muscle; *Bull. Jpn. Soc Sci. Fish.* 5 95-102
- George J C 1962 A histophysiological study of the red and white muscles of the mackerel; *Am. Midl. Nat.* 68 487-494

- George J C and Berger A C 1966 *Avian myology* (New York : Academic Press)
- George J C and Bokdawala F D 1964 Cellular organization and fat utilization in fish muscle ; *J. Anim. Morphol. Physiol.* **11** 124-132
- Kun E and Abood L G 1949 Colorimetric estimation of succinic dehydrogenase by Triphenyl tetrazolium chloride ; *Science* **109** 144-146
- Lawrie R A 1952 Biochemical difference between red and white muscle ; *Nature* **170** 122
- Love R M 1970 *The chemical biology of fishes* (London and New York : Academic Press)
- Modigh M and Tota B 1975 Mitochondrial respiration in the ventricular myocardium and in the white and deep red myotomal muscles of the juvenile tuna fish (*Thunnus thynnus* L.); *Acta Physiol. Scand.* **93** 289-294
- Nayeemunisa and Rao K P 1972 Effects of thermal acclimation in the lipid metabolism in the earthworm ; *Lampito mauritii*—A method for lipid estimation ; *Comp. Biochem. Physiol.* **B42** 167-173
- P'shawikar S D 1961 *A study of the phosphates, phosphatases and certain inorganic ions in the pectoralis muscle of some birds with special reference to that of the pigeon.* Doctoral Thesis M.S. University of Baroda, Baroda, India
- Rayner M D and Keenan M J 1967 Role of red and white muscles in the swimming of skip-jack tuna ; *Nature (London)* **214** 392-393
- Seifter S, Dayton S, Novic B and Muntwyler E 1950 The estimation of glycogen with the Anthrone reagent ; *Arch. Biochem.* **25** 191-200
- Talesara C L and Vasdev Narang 1979 A comparative study of myofibrillar ATPase (*m*-ATPase) and succinic dehydrogenase (SDH) activities in certain specialised muscles of rat (*Rattus norvegicus*) from various representative regions ; *Indian J. Exp. Biol.* **17** 219-221
- Tappan DU and Raynaferjee B 1957 Tissue pigment manifestations of adaptation to high altitudes ; *Am. J. Physiol.* **190** 99-103
- West E S, Todd W R, Manson H S and Bruggen J T V 1956 *A text book of Biochemistry.* (New York: Macmillan Co.)
- Wong 1923 Estimation of proteins in blood plasma ; *J. Biol. Chem.* **55** 427
- Zama K 1963 Studies on the phospholipids of aquatic animals ; *Mem. Fac. Fish. Hokkaido Univ.* **11** pp 73