CHAPTER 3

CHANGES IN FAT AND WATER CONTENTS IN THE RED AND WHITE MUSCLES OF *HILSA ILISHA* AND *HILSA TOLI* DURING DIFFERENT STAGES OF LIFE CYCLE

The composition of muscular tissue of fishes is affected by various factors such as the availability of food, age, migrations and spawning cycles. Migratory fishes such as the salmon and rainbow trout are known to be fasting during their long journey from the sea to the river and the energy reserves in the body are utilized for development and maturation of gonads, as well as for the sustained muscular activity involved.The depletion of the energy reserves of migrating salmon has been studied by several investigators and the earlier studies have been reviewed by Drummond and Black (1960).

That stored fat serves as the main source of energy for the muscular activity during fasting migrations and for the building up of reproductive organs,is well established (Miescher-Rusch, 1883; Greene, 1913, 1919; Pentagov *et al.*, 1928; Dunston, 1956). In the sockeye salmon, *Oncorhynchus nerka*, a reduction of 70% and 80% fat in the male and female respectively was recorded on completion of migration (Idler and Bitners, 1958; Idler and Clemens, 1959). Apart from fishes, storage of large amounts of fat by migratory birds and its reduction during migrat-

ion have been shown by several authors (Wolfson, 1954; Odum and Perkinson, 1951; Odum and Connel, 1956; Naik, 1963; Vallyathan, 1963) and the problem of fat metabolism in migration has been discussed by George and Berger (1966). Similarly in the migratory locusts, Weis-Fogh (1952) showed that at least 2/3 of the energy expended during flight was derived from fat.

In fishes such as salmon and herring, the fat is stored mainly in the muscle, while in the case of cod, storage of fat takes place mainly in the liver (Bailey, 1952). Greene (1913) had shown that in the king salmon the tissues primarily concerned with the premigratory storage of fat are the muscles and the intermuscular connective tissue.

In the light of the above mentioned investigations, the present study was undertaken with a view to understand the histochemical localization of fat in the red and white muscles of H.ilisha which is known to migrate from sea to river for the purpose of spawning, without feeding on the way. It was also thought worthwhile to determine quantitatively the variations in fat and water contents of the above mentioned muscles in different regions of the body of the same fish. Similar studies were carried out on a closely related non-migratory marine species, H.toli, to find out whether the changes that take place in H.ilisha are due to migration or as a result of maturation.

MATERIALS AND METHODS

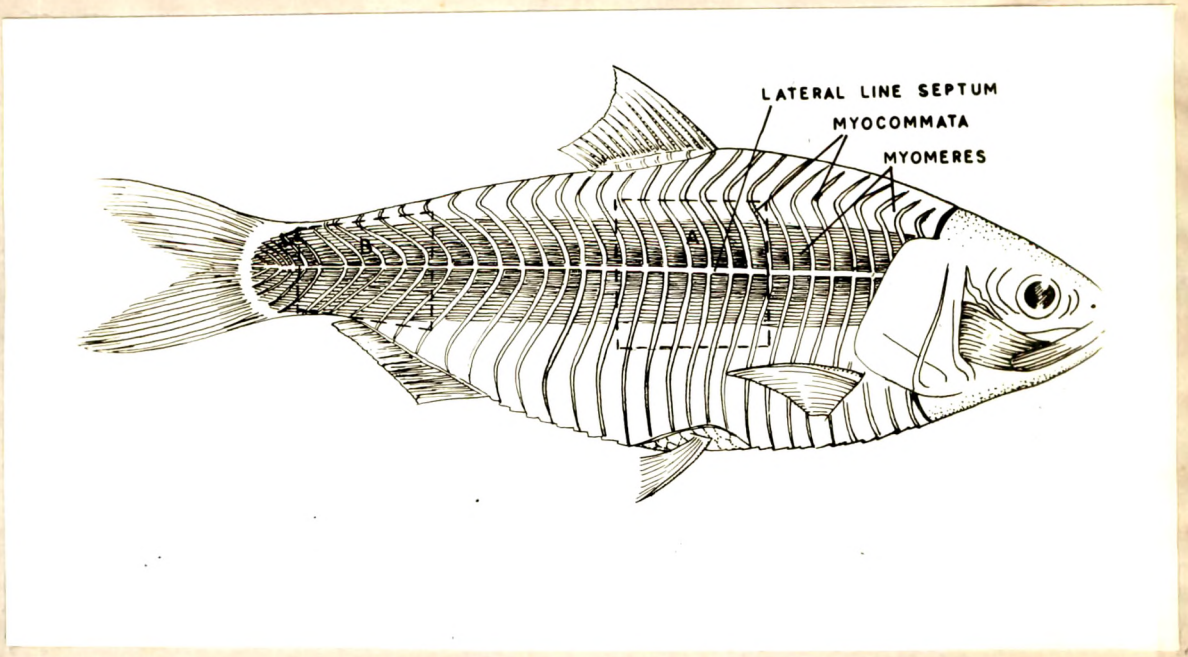
H.ilisha and H.toli of different stages of maturity were used in the present investigation. They were collected as described in the previous chapter.

The various stages of maturity of gonads were determined according to the classification of Gokhale (1953) and Bower (1954).

Fishes were sacrificed immediately after collection from the nets and pieces of the lateral muscle from the middle (A) and tail (B) regions (Text fig. 1) were fixed in 10% calcium formol (Baker, 1946) for 24 hours, post-chromed in 30% potassium dichromate solution for 6 - 7 hours, washed overnight in running tap water and embedded in 15% gelatin. Sections of 20µ thickness were cut on a freezing microtome and stained with Sudan Black B to study the distribution of lipids. For neutral lipids, sections were stained with Fettrot 7B (Pearse, 1960), Oil Red O (Pearse, 1960)and Nile blue sulphate (Gurr, 1962). The Acid haematein method (Pearse, 1960) was employed for the demonstration of phospholipids.

Pieces of the lateral muscle from the middle and the tail regions were taken out from each fish, wrapped in polyethylene bags, kept in ice and brought to the laboratory for the quantitative studies.

After removal of the skin and bones from all the



Text Fig. 1 Showing the two different anatomical regions (A & B) along the body of the fish from which samples were taken for the various quantitative estimations.

samples, the red and white muscles of both the regions were separated along with the intermuscular connective tissue and myocommata and weighed. The layer of fat, if present between the skin and the muscle region, was completely excluded.

The tissues were then dehydrated to constant weight in a hot air oven at 100°C to estimate the percentage of water. The fat content of the dehydrated tissue was estimated by the Soxhlet extraction method, using 1:1 alcohol-ether mixture.

In the case of juveniles of H.ilisha, the quantity of fat could not be assessed in the different regions of the body, owing to their small size. Instead, the red and white muscles were separated from along the entire length of the fish. Besides, the material had to be pooled from 8 to 10 individual fishes for each assay.

RESULTS

The lateral line muscle of Hilsa consists of a superficial red muscle strip along the lateral line and a deep seated white muscle region, the two being separated by lamina of connective tissue. The red as well as white muscles are traversed along their entire length by a septum - the lateral line septum - which extends from the vertebral column to the outer skin, and divides them into a more or less equal dorsal and ventral half. Transversely, the lateral line muscle (red and white) is divided into a

number of myomeres by connective tissue partitions, the myocommata or myosepta (Text Fig. 1). The myocommata, the lateral line septum and the fibrous connective tissue between the red and white muscle regions were found to show modifications according to the stage of maturity, since they are the main sites for fat storage.

A region intermediate between the red and white muscle regions, comprising of an admixture of red and white fibres is totally lacking in both species of Hilsa.

Histochemical observations :

In the various stages of maturity, in the fibres of the red muscle region, intracellularly the mitochondria showed positive staining with all the fat stains used. The mitochondria which stain for fat are numerous and bigger at the periphery of the fibres (Figs. 1 & 2). On the other hand in the white muscle region, little or no intracellular fat could be demonstrated. In both the muscle regions the extracellular fat cells if present, showed positive staining with neutral fat stains.

Juveniles of H. ilisha collected from the freshwater zone :

The fibres of red as well as white muscle regions were found to be arranged compactly with little interfascicular connective tissue (Fig. 3). The myocommata and connective tissue lamina which were thinly represented at this stage of maturity contained only very few fat globules,

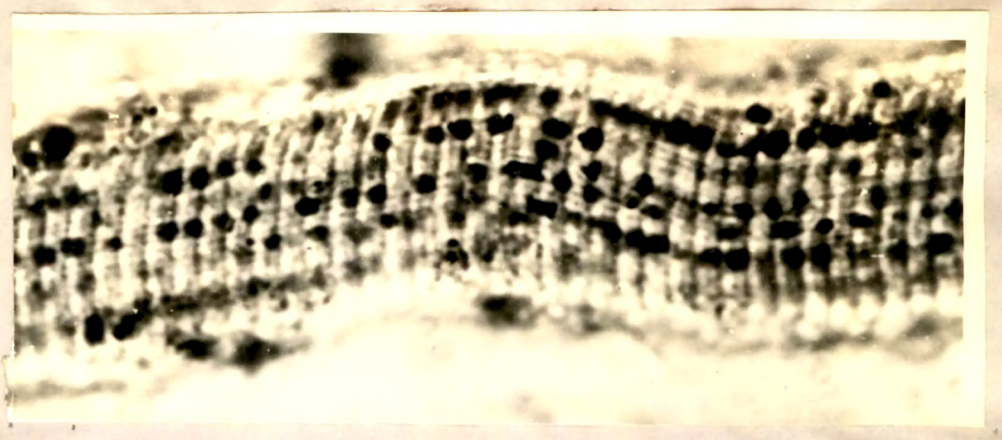


Fig. 1. Longitudinal section (L. S.) of red fibre stained with Sudan Black B to show the localization of fat.1,800 X.

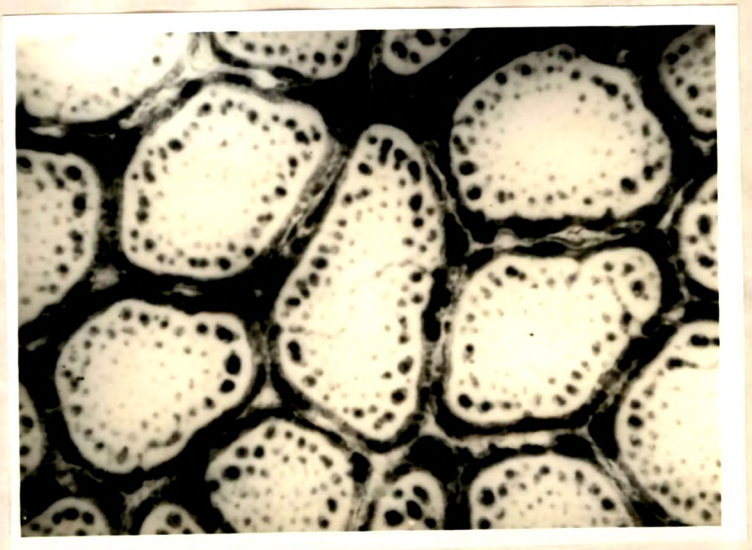


Fig. 2. Transverse section (T. S.) of red fibres stained with Sudan Black B to show the localization of fat. The mitochondria are more numerous and larger at the periphery of fibres. 567 X.

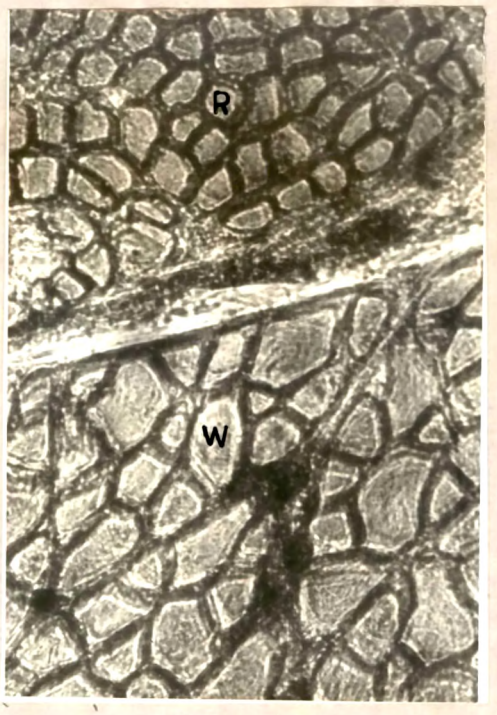


Fig. 3. The red (R) and white (W) fibres (T. S.) of juveniles of H.ilisha stained with Nile blue sulphate. Note the compact arrangement of fibres. 144 X.

and they were of very small size.

Immature H. ilisha from sea :

Great variations were noted in the deposition of fat at this stage, in accordance with the changes in gonadal development. With respect to fishes of IIIrd stage it was found that as compared to the juveniles, the areas of interfascicular connective tissue, myocommata and connective tissue lamina between the red and white muscle regions were more enlarged and contained a number of relatively uniform sized, fat cells (Figs.4 & 5). The presence of fat globules was noted intracellularly in many fibres of the red muscle region.

In H.ilisha of IVth stage, although similar results were met with as above, some striking differences were noted. A fat layer of 1.2 to 1.5 mm. thickness was observed around the body between the skin and muscle region. So also, the concentration of fat in both red and white muscle regions was seen to be enormously increased (Figs. 6 & 7). The neighbouring fasciculi in both the muscle regions and the individual fibres also in the case of red muscle region were found to be widely separated by connective tissue laden with comparatively larger fat cells, so much so that they appeared to be completely embedded in a mass of fatty tissue (Fig. 6). The intracellular fat globules in the red fibres showed much increase in number (Fig. 8).

In contrast, in the white muscle region, only

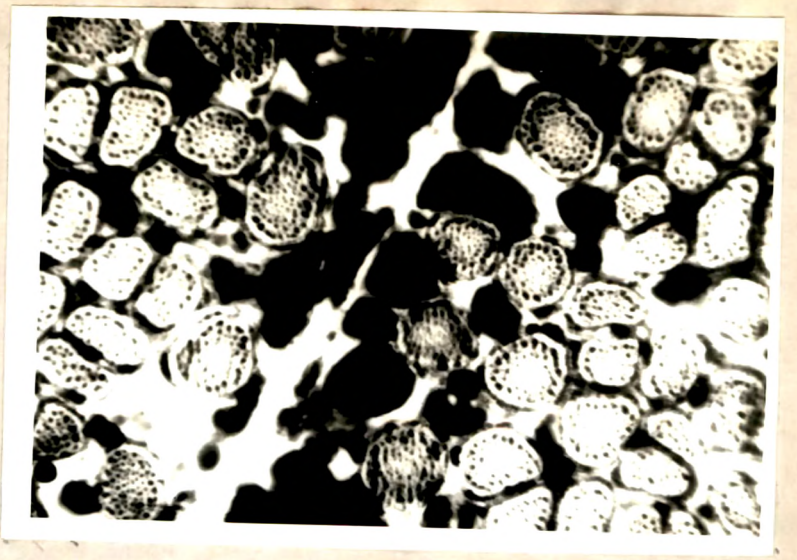


Fig. 4

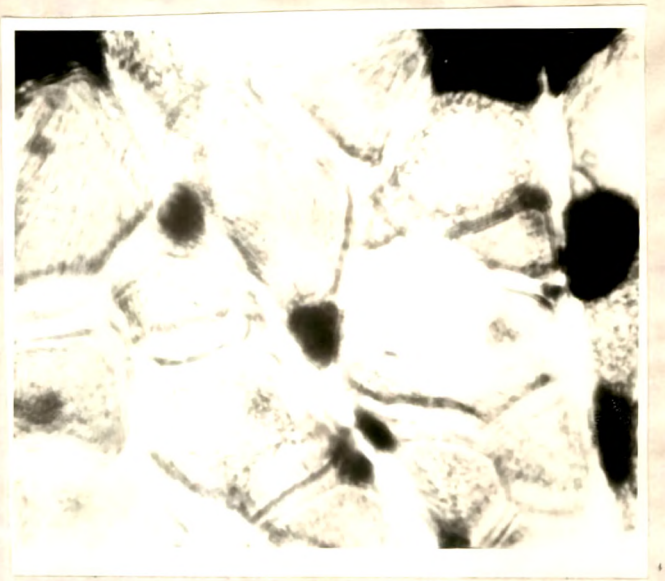


Fig. 5

Figs. 4 & 5. Red and white muscle regions (T. S.) of immature (Stage III) H. ilisha stained with Sudan Black B. Note the interfascicular and interfibral fat deposition. 225 X.

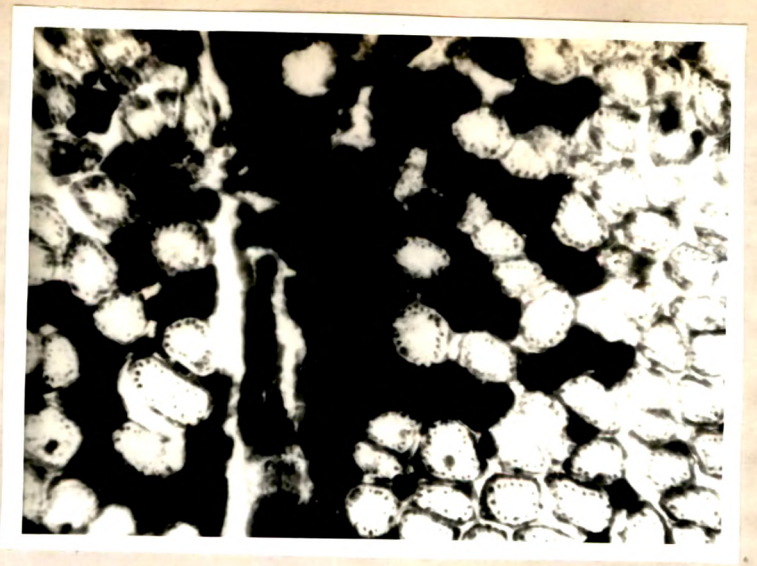


Fig. 6

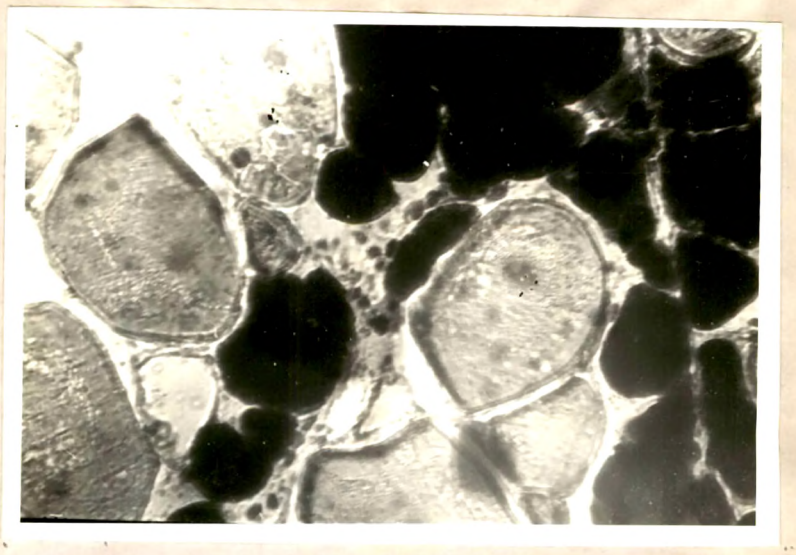


Fig. 7

Figs. 6 & 7. Red and white muscle regions (T. S.) of immature (Stage IV) H. ilisha stained with Sudan Black B. Note the enormous interfascicular and interfibral storage of fat. 225 X.

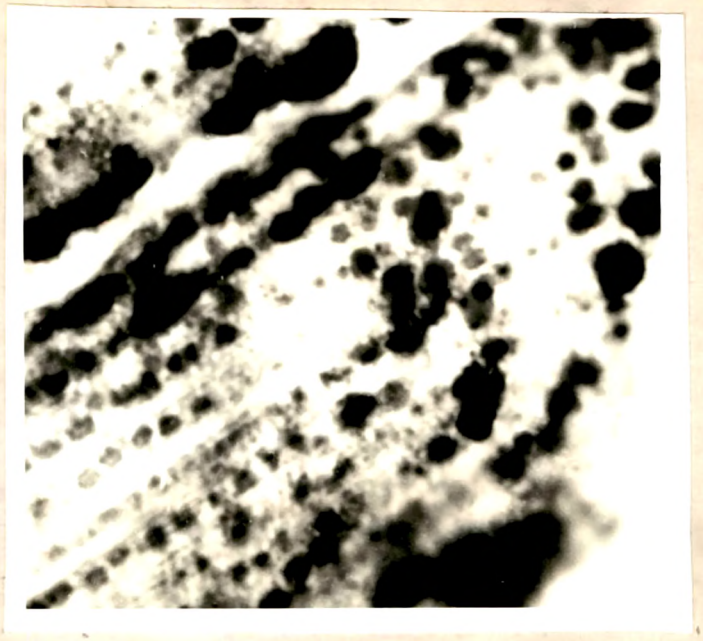


Fig. 8

L. S. of a red fibre (part of another fibre seen at top left) of immature H. ilisha stained with Fettrot 7B. Note the intracellular fat globules. 1,800 X.

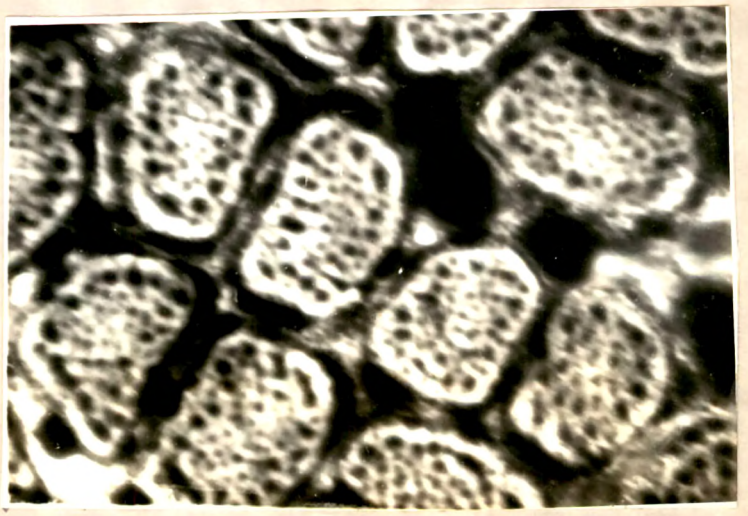


Fig. 9

T. S. of red fibres of immature H. ilisha stained with Sudan Black B to show the localization of fat. 567 X.

traces of fat could be located intracellularly, whereas the amount of interfascicular connective tissue loaded with fat cells showed a remarkable increase, even greater than that of the red muscle region (Fig. 7).

Mature H. ilisha from river mouth :

The results obtained were more or less intermediate between those of the immature (IVth stage) and the spent stages. The concentration of fat in the interfascicular connective tissue, myocommata and connective tissue lamina, though abundant, a considerable reduction was observed in the number and size of fat cells in the red as well as white muscles (Figs. 10 & 11). This reduction was more pronounced in the latter muscle, where the interfascicular connective tissue was much lesser than in immature fishes of IVth stage of maturity. Intracellularly in many red fibres small fat globules were seen at this stage also (Fig. 12).

Mature and spent H. ilisha from freshwater zone :

This stage was characterized by the disappearance of the fat layer observed between the skin and the muscle, in H.ilisha of IVth stage of maturity. The localization of fat in both the muscle regions was in sharp contrast to that of fishes from the sea or river mouth(Figs. 14, 15, 16, 17, 18). Although the fat cells in the interfascicular connective tissue, myocommata, and connective tissue lamina were very much reduced, a number of fat cells could be seen at some places in the red muscle region. Around the lateral line

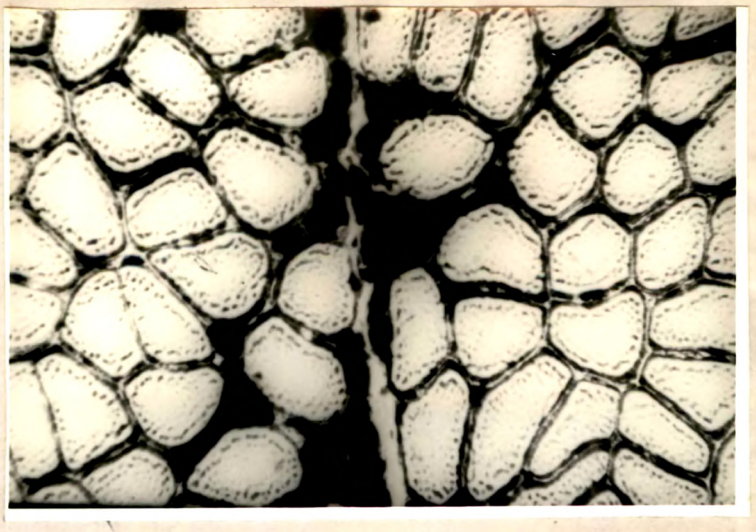


Fig. 10. Red fibres (T. S.) of mature H. ilisha collected from river mouth. Note the reduction of interfascicular and interfibral fat. Sudan Black B. 225 X.

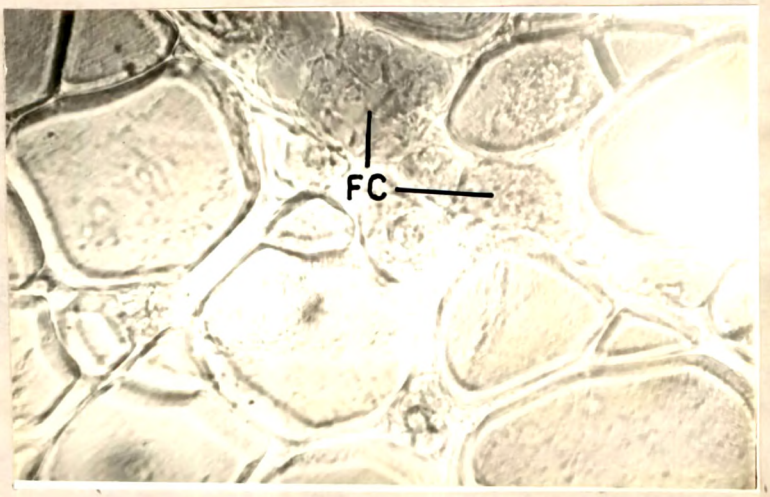


Fig. 11. White fibres (T. S.) of mature H. ilisha collected from river mouth stained with Nile blue sulphate. Note the reduction of interfascicular and interfibral fat. 225 X.
F.C.-fat cell.

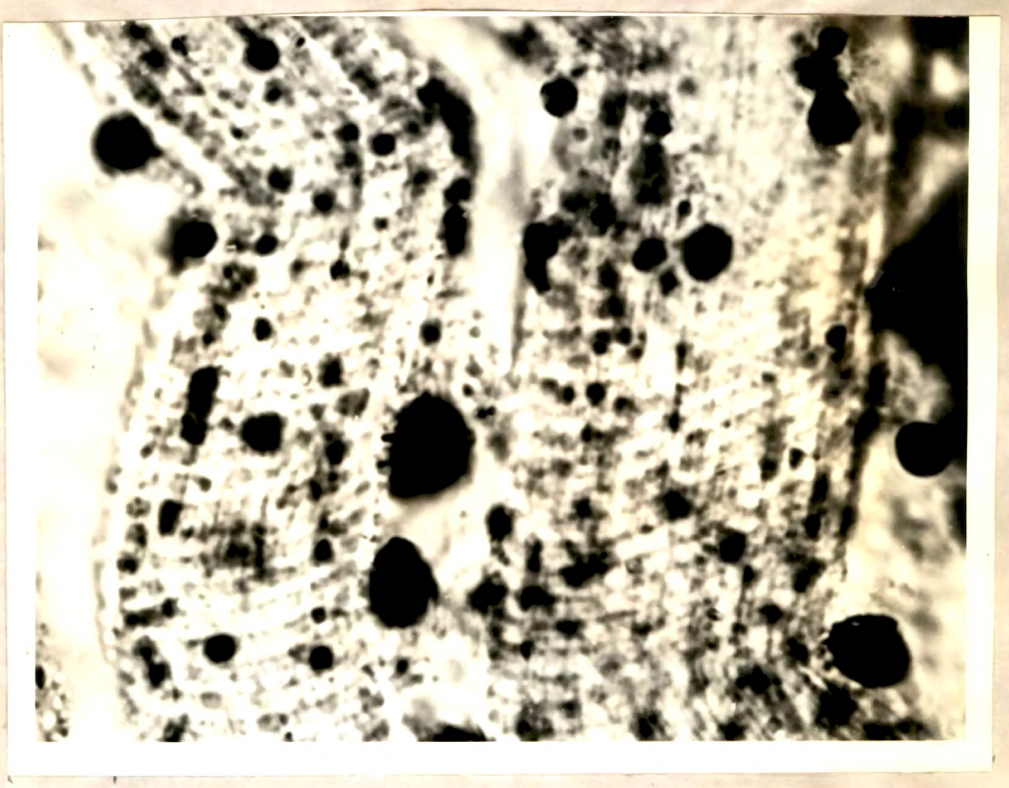


Fig. 12. L. S. of red fibres of mature H. ilisha collected from river mouth, stained with Fettrot 7B. Note the reduction of intracellular fat globules. 1,800 X.

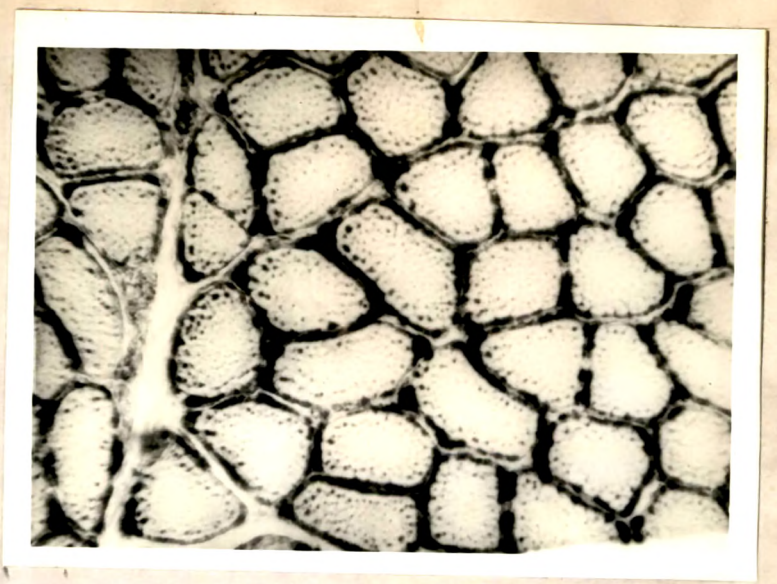


Fig. 14

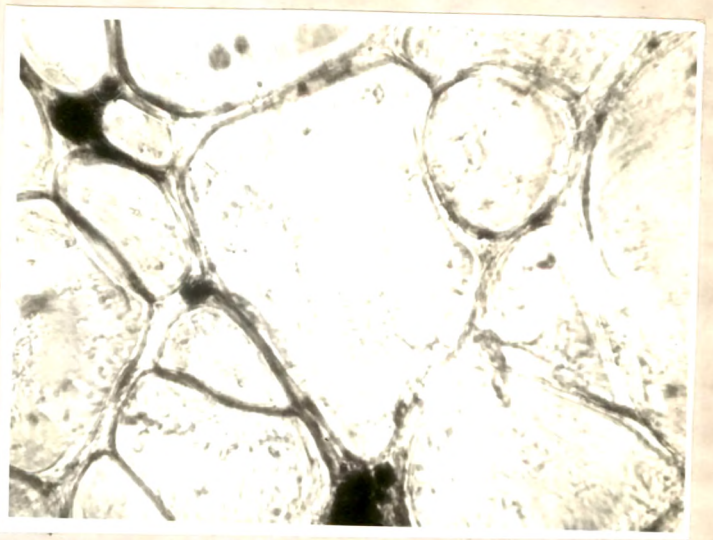


Fig. 15

Figs. 14 & 15. Red and white fibres (T. S.) of mature H. ilisha collected from freshwater zone, stained with Sudan Black B. Note the disappearance of interfascicular fat in the red muscle. 225 X.

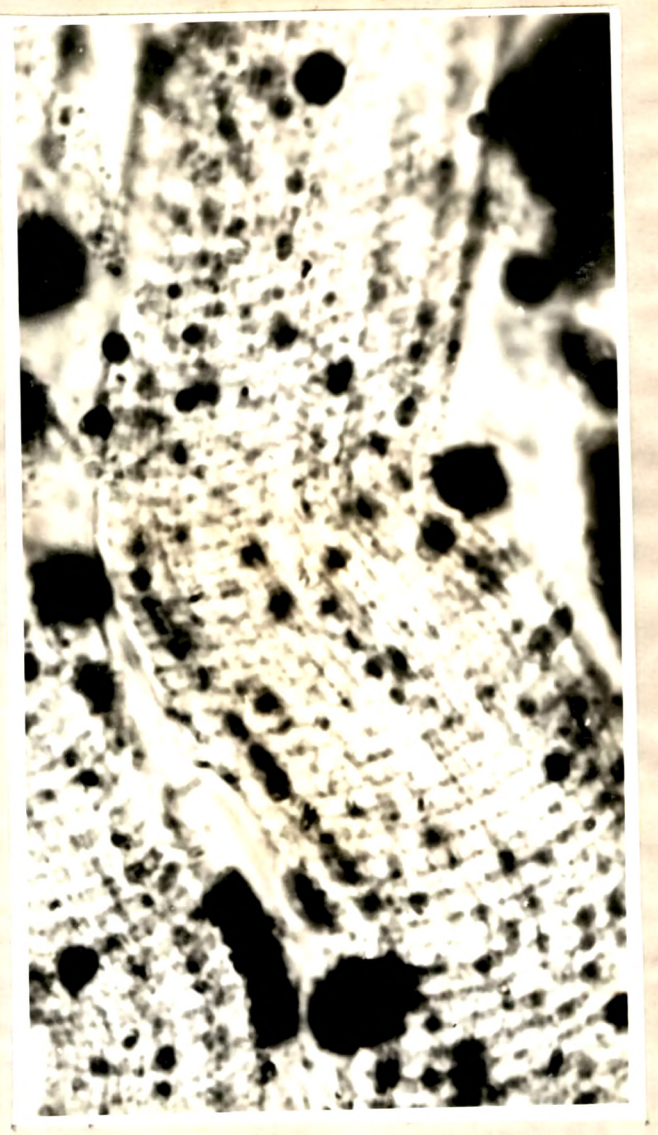


Fig. 16

L. S. of red fibres of mature H. ilisha collected from fresh-water zone, stained with Fettrot 7B. Note the reduction in the number of intracellular fat globules. 1,800 X.

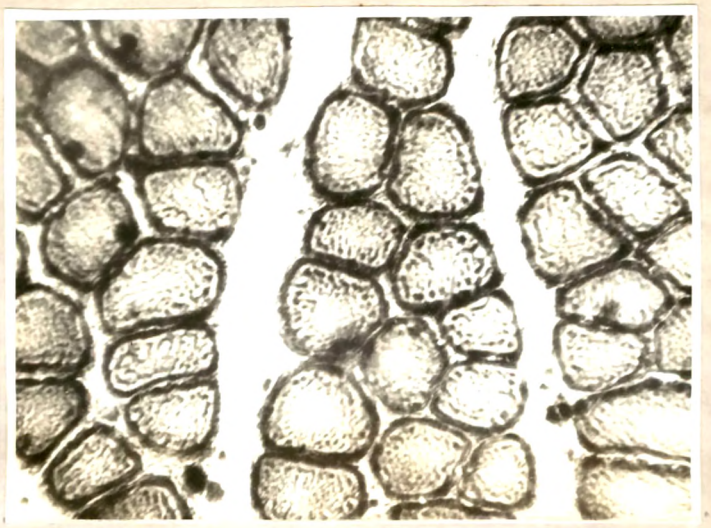


Fig. 17. Red fibres (T. S.) of spent H. ilisha collected from the freshwater zone, stained with Sudan Black B. showing the disappearance of interfascicular and interfibral fat. 225 X.

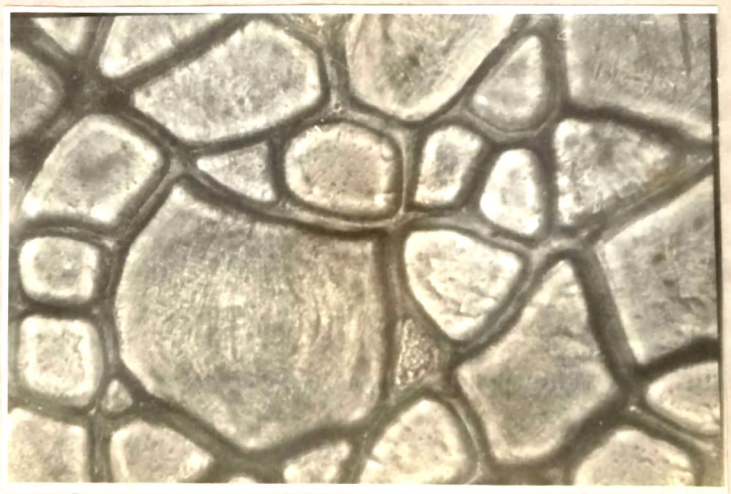


Fig.18. White fibres (T. S.) of spent H. ilisha stained with Nile blue sulphate showing the disappearance of interfascicular and interfibral fat. 225 X.

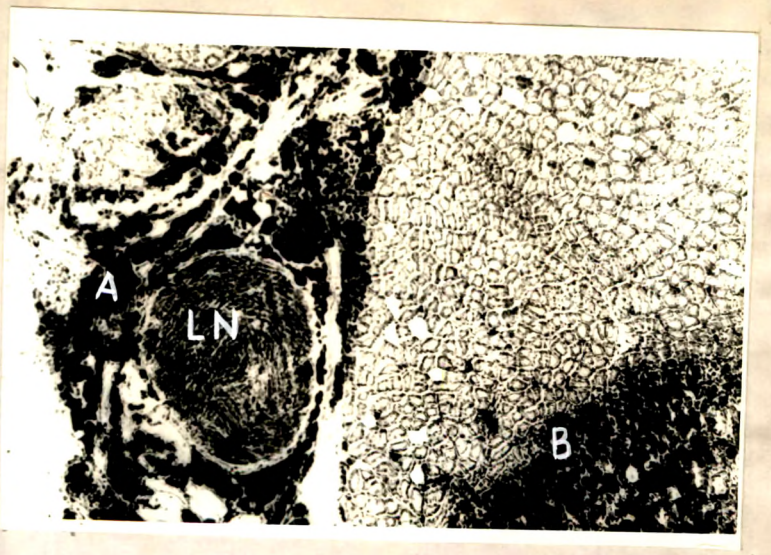


Fig. 19

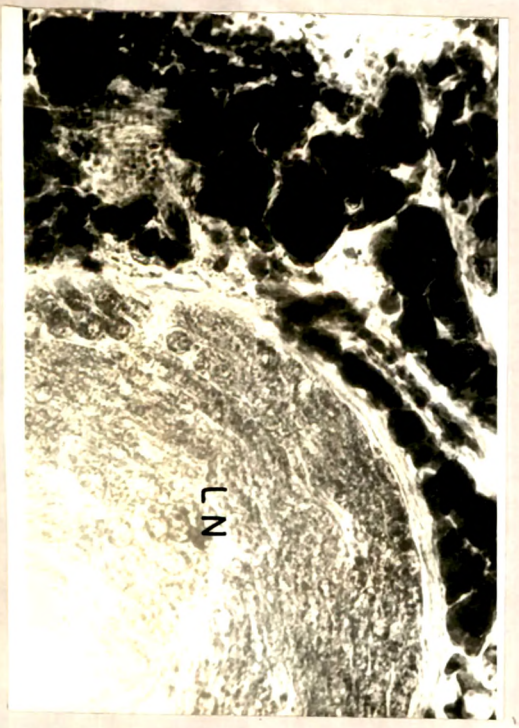


Fig. 20

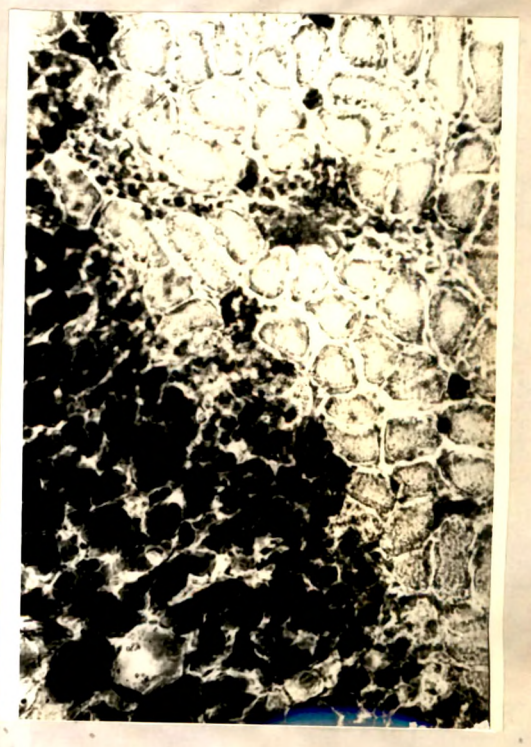


Fig. 21

Fig. 19. Photomicrograph showing fat cells around the lateral line nerve (L.N.) (region A) and at the sites of degeneration of red muscle fibres (region B) (T. S.) in mature H. ilisha collected from the freshwater zone. Fettrot 7B. 53 X.

Figs. 20 & 21. Magnified views of regions A and B. 225 X.

nerve, blood vessels and at sites of degeneration of red muscle fibres (Chapter 2) the fat cells were abundant (Figs. 19, 20, 21). The intracellular fat globules(in the red fibres), which were plenty in fishes of IVth and Vth stages of maturity, showed much reduction (Fig. 16).

In the fibres of the white muscle region, only traces of intracellular fat could be detected as in other stages. The extracellular fat if present was reduced to small droplets, restricted to places where many fasciculi group together (Fig. 15).

HILSA TOLI

Immature specimens of IIIrd and IVth stages of maturity showed the same staining pattern as observed in H.ilisha of IIIrd stage of gonadal development from sea. Mature (stage V & VI) and spent fishes from sea resembled the mature and spent H.ilisha respectively from the river in the disposition of neutral fat in the interfasicular connective tissue and in the staining revealing mitochondial fat. However, in the case of mature and spent H.toli collected from river, the interfascicular connective tissue laden with neutral fat cells was found to be in greater abundance in the red as well as white muscle regions as compared to fishes of the same stage of maturity from the sea.

Quantitative studies :

The percentage of fat and water contents in the red white muscles of middle and tail regions of H.ilisha

Table I. Fat and Water contents in the red muscle of H. ilisha in various stages of maturity.

Place of collection	Stage of gonad	Sex	Fat (Percent wet weight)		Water (Percent wet weight)	
			Mid.region	Tail region	Mid.region	Tail region
Sea	Immature Group I	F.(3)	20.58±2.2	5.57±1.2	61.82±2.64	72.5±1.15
		M.(1)	22.27	6.39	59.23	72.35
	Immature Group II	F.(3)	37.23±2.24	18.83±1.74	46.90±2.44	60.91±1.9
		M.(3)	34.53±1.83	15.22±2.13	48.01±2.02	63.69±1.41
Bhadbhut (River mouth)	Mature	F.(4)	19.14±3.46	8.16±1.73	64.12±3.8	71.49±1.96
		M.(4)	24.18±1.9	9.09±0.86	59.59±2.14	69.87±1.82
Makthampore & Zadeswar (Fresh-water zone)	Mature	F.(4)	12.01±2.06	5.46±1.08	68.11±3.02	73.44±1.73
		M.(4)	17.99±1.45	6.14±1.11	63.83±2.13	72.84±1.42
	Spent	F.(3)	10.05±1.58	3.78±0.81	71.85±1.73	76.02±0.45
		M.(3)	15.48±1.41	5.55±1.03	67.32±1.94	73.82±1.50
	Juveniles (2 groups)		av=7.754		av= 76.46	

' ± ' Standard deviation. Figures in parentheses indicate the number of fishes analysed.

Table II. Fat and Water contents in the white muscle of H. ilisha in various stages of maturity.

Place of collection	Stage of gonad	Sex	Fat (Percent wet weight)		Water (Percent wet weight)	
			Mid.region	Tail region	Mid.regiom	Tail region
Sea	Immature Group I	F.(3)	4.83±0.86	2.55±0.77	73.56±1.60	75.33±1.70
		M.(1)	3.23	1.72	75.98	77.62
	Immature Group II	F.(3)	28.53±3.46	10.59±2.50	53.44±3.30	68.09±2.64
		M.(3)	25.65±2.45	7.97±1.73	56.68±2.45	70.05±1,60
Bhadbhut (River mouth)	Mature	F.(4)	5.91±1.41	1.89±0.34	74.34±2.45	77.88±1.73
		M.(4)	8.29±1.66	2.71±0.12	72.89±2.17	76.89±1.41
Makthampore & Zadeswar (Freshwater zone)	Mature	F.(4)	1.92±0.19	1.32±0.33	76.42±1.40	79.08±2.85
		M.(4)	4.06±1.26	1.78±0.21	74.30±1.74	76.52±1.26
	Spent	F.(3)	1.48±0.57	1.02±0.31	77.99±1.40	78.78±1.70
		M.(3)	3.85±0.63	1.81±0.14	74.42±1.22	76.56±1.12
	Juveniles (2 groups)		av.= 0.772		av.= 81.98	

'±8 Standard deviation. Figures in parentheses indicate the number of fishes analysed.

Table III. Fat and Water contents in the red muscle of *H. toli* in various stages of maturity.

Place of collection	Stage of gonad	Sex	Fat (Percent wet weight)		Water (Percent wet weight)	
			Mid.region	Tail region	Mid.region	Tail region
Sea	Immature	F.(4)	18.31±2.03	5.34±1.07	63.01±1.83	72.27±1.17
		M.(3)	17.52±1.06	5.96±0.48	65.67±1.40	70.02±1.35
	Mature	F.(4)	12.05±2.23	3.02±0.24	69.57±2.68	74.19±1.52
		M.(3)	15.15±1.01	3.69±0.38	68.02±1.71	72.59±1.12
	Spent	F.(3)	10.87±1.70	2.45±0.44	71.25±1.68	76.62±1.22
		M.(2)	13.21	3.43	69.50	74.14
Bhadbhut (River)	Immature	F.(2)	20.54	6.56	63.12	72.42
		M.(1)	19.48	6.52	64.56	70.08
	Mature	F.(3)	15.58±1.15	5.36±0.53	67.57±1.80	73.12±1.31
		M.(1)	16.65	5.73	66.83	72.52
	Spent	F.(1)	14.24	4.59	68.45	74.76
		M.	-	-	-	-

' ± ' Standard deviation. Figures in parentheses indicate the number of fishes analysed.

Table IV. Fat and Water contents in the white muscle of H. toli in various stages of maturity.

Place of collection	Stage of gonad	Sex	Fat (Percent wet weight)		Water (Percent wet weight)	
			Mid.region	Tail region	Mid.region	Tail region
Sea	Immature	F.(4)	4.46±1.23	2.20±0.29	73.09±1.85	76.31±1.41
		M.(3)	4.63±0.74	2.27±0.22	71.26±1.72	75.62±1.17
	Mature	F.(4)	1.73±0.32	1.01±0.14	76.96±1.48	77.44±1.64
		M.(3)	2.58±0.27	1.48±0.17	73.96±1.54	76.79±1.21
	Spent	F.(3)	1.38±0.38	0.78±0.05	77.55±1.63	78.68±1.40
		M.(2)	2.15	1.14	76.45	77.98
Bhadbhut (River)	Immature	F.(2)	5.64	2.35	73.05	76.42
		M.(1)	5.26	2.02	72.53	75.03
	Mature	F.(3)	4.48±0.98	1.42±0.28	74.83±2.23	77.25±1.44
		M.(1)	4.46	1.95	73.52	75.03
	Spent	F.(1)	3.21	1.91	75.69	76.25
		M.	-	-	-	-

' ± ' Standard deviation. Figures in parentheses indicate the number of fishes analysed.

Table V. Percentage reduction of fat in H.ilisha and H.toli during different stages of maturity.

Species	Place of collection and stage of gonad	Sex	RED MUSCLE			WHITE MUSCLE		
			Mid. region	Tail region	Average	Mid. region	Tail region	Average
H. ILISHA	Bhadbhut (Rivermouth) Mature	F.	48.6	56.69	52.64	78.93	82.15	80.59
		M.	29.97	40.33	35.15	68.08	66.oo	67.04
	Makthampore & Zadeswar Mature & Spent	F.	64.74	71.01	69.38	93.33	87.46	90.40
		M.	47.90	59.61	53.75	84.22	77.64	80.93
		F.	73.00	79.91	76.45	94.73	90.18	92.45
		M.	55.15	63.56	59.37	85.14	77.29	81.22
H. TOLI	Sea Mature	F.	34.22	43.32	38.77	61.24	54.28	57.76
		M.	13.54	37.97	27.75	44.16	35.25	39.71
	Sea Spent	F.	40.63	54.12	47.38	69.02	65.46	67.24
		M.	24.60	42.46	33.53	53.70	50.23	51.96

and H.toli during various stages of gonadal maturity are presented in Tables I to IV.

In all the stages of H.ilisha and H.toli, the red muscle of the middle region possessed a much higher concentration of fat and lesser quantity of water than that of the tail region. In the white muscle, a distinct difference between the two regions was noted in the immature H.ilisha (Group II) from the sea and mature H.ilisha from river mouth. The rest of the stages did not show a very significant difference.

In immature H.toli, there was no difference between the sexes as regards fat storage - 7.5% fat in females (Red muscle = 11.84%, White muscle = 3.33%) and 7.59% fat in males (Red muscle = 11.74%, White muscle = 3.45%). Immature H.ilisha from sea (Group II) were seen to store three times more fat than immature H.toli and a difference in the fat content of the two sexes were also noted - fat content in females 23.8% (Red muscle = 28.03%, White muscle = 19.56%) and in males 20.85% (Red muscle = 24.88%, White muscle = 16.8%). Thus the fat store in the female was found to be greater than that in the male.

During the spawning migration of H.ilisha, a gradual reduction of fat and a simultaneous increase in water content were observed. The percentage changes in the different stages of both the fishes are presented in Table V. On completion of migration and the spawning a reduction

of 84.45% fat (Red muscle = 76.4%; White muscle = 92%) in females and 70.3% fat (Red muscle = 59.4%; White muscle =81.22%) in males took place. H.toli were also found not to feed during the spawning season and on maturation and spawning they showed a reduction of 48 - 57% fat in females (Red muscle = 38.7 - 47.16%; White muscle = 57.76 - 67.24%) and 32 -42% (Red muscle = 25.76 - 33.53%; White muscle = 39.71 - 51.96%) in males. Thus females of both the species were found to utilize more stored fat than males.

DISCUSSION

From the results obtained it is evident that the intra- and extracellular fat content was considerably higher in the fibres of the red muscle than the white. The fat-loaded red fibres have been shown to be well adapted for slow contractions, while the white fibres with relatively less fat for fast contractions of short duration (Boddeke et al., 1959; George, 1962; George and Bokdawala, 1964). In the parietal muscle of the Atlantic hagfish, Myxine glutinosa (L.) Flood (1962, 1965) reported the presence of fibres intermediate in nature with regard to the concentration of fat, besides the typically fast and slow fibres. They are similar to the intermediate fibres reported in bird muscle (George and Berger, 1966) and in mammalian muscle (Stein and Padykula, 1962), but such fibres could not be observed in the lateral muscles of both the fishes studied. Bone (1966) found that the fat content of the slow red

fibres of dogfish was 4 - 5 times that in the white fibres and reduced considerably after long periods of sustained activity. He also furnished evidence to show that only red fibres were active during slow swimming of the spinal dogfish, whereas during vigorous movements, the white fibres took active part; thereby contradicting the view of Braekkan (1956) that the function of red muscle is more or less similar to that of the liver.

A relatively higher fat store noted in the middle region of the red as well as white muscles than in the tail region, in all the stages of maturity is in accord with the results of Thurston (1957), Ono et al. (1959), Mannan et al. (1961) and Bokdawala (Unpublished). This may be due to the higher utilization of fat to bring about the quick lashing movements of the tail. The water content on the other hand was inversely related to the fat content. Jacquot and Creach (1950) reported that the excess fat was acquired at the cost of the water in the tissue. Similarly in the herring, Brandes (1954) has shown the existence of a close relationship between the two, so that the water content is also an indirect index of the fat content.

The composition of fat in fishes is known to vary with many factors, viz. size, sex, stage of maturity, season, locality etc. Variations in the fat content based on some of these factors have been demonstrated by Clark and Almy (1918) in shad, Lovern (1938) in the Atlantic herring and

Hart et al. (1940) in the Pacific herring. According to Thurston (1957) the variations with sex and size was small in comparison with that due to sexual maturity. In the present study, a reduction of fat in both male and female specimens of H.toli could be observed on maturation and spawning. This reduction could be attributed to the lack of feeding, since their stomachs on examination were found to be empty during the spawning season. The higher percentage of fat observed in all the stages of H.toli collected from the river mouth may be due to the availability of more food there.

The deposition of fat in the immature H.ilisha from sea (Group II) was about three times higher than that in H.toli of the same stage of maturity. The fat store was in abundance in the myocommata, connective tissue lamina between red and white muscles, lateral line septum, and interfascicular connective tissue in the form of neutral fat cells; and inside the red fibres as neutral fat globules. Miescher (1898) considered that intracellular deposition takes place by a process of fatty degeneration. Later studies by Greene (1913) showed that fat was deposited by 'fatty infiltration' or absorption from the blood and lymph.

The gradual reduction in fat in the red as well as white muscles of H.ilisha could be ascribed to the utilization of lipids as energy source. Evidence in favour of the utilization of fat for energy by the red muscle fibres of fishes during prolonged muscular activity is provided

by the fact that the oxidation of fatty acids takes place at a much higher rate in the red than in the white muscle (Bilinski, 1963; George and Bokdawala, 1964). However, the reduction of considerable amounts of extracellular and interfascicular fat stored in the white muscle region during pre-migratory stage, indicates that fat is used as fuel in the white muscle as well. George (1962) has suggested that the white muscle of the mackerel when subjected to prolonged activity could utilize as energy fuel, the fatty acids derived from the extracellular fat stored within the muscle, adipose tissue and liver. Young and Price (1961) had obtained similar results with regard to dog muscle. Neverthless it cannot be considered entirely due to its utilization there, but more due to its transport to other parts of the body. This view is supported by the fact that only traces of fat could be demonstrated intracellularly in the white fibres. Although the energy expended for swimming would be same for both sexes, the females were found to utilize more fat than males. This difference has been attributed to the greater utilization of fat by females for the development of the gonads (Idler and Bitners, 1958). The greater increase in the weight of ovary than of the testis is also in accordance with the above mentioned view. Black (1958) postulated that the energy for the development and maturation of gonads as well as for muscular energy must be provided through the metabolism of fat and protein reserves in the body. Drummond and Black (1960) have reiterated that

in the fish muscle the store of fat serves as a large source of energy reserve. According to Fontaine and Hatey (1953) gluconeogenesis from lipids and perhaps also from aminoacids is responsible for the carbohydrates required in muscular activity during migration.

That glucose can increase the rate of incorporation of FFA into triglycerides of skeletal muscle has been shown by Eaton and Steinberg (as cited by Steinberg and Vaughan, 1963). Hence the extra stores of glucose accumulated at the time of feeding, may be utilized to build up the endogenous lipids in muscle which can be subsequently drawn up during the fasting state. At the time of fasting, the supply of glucose being at a minimum, the rate of release of fatty acids from ester form, exceeds the rate at which they can be re-esterified (Steinberg and Vaughan, 1963), thereby liberating FFA into the serum. Therefore during this period, the serum FFA constitute the major source of substrate for most of the tissues of the body including the skeletal muscle. In exercised pigeon, George and Vallyathan (1964) have shown the transport of fat in the form of FFA from liver and adipose tissue through the blood to the muscle.

The rise in serum FFA level as a result of the mobilization of fat from fat depots has been shown to be influenced by the thyroid and pituitary hormones (Steinberg and Vaughan, 1963). In maturing H.ilisha from sea and river

mouth, thyroid and pituitary were found to exhibit a marked increase in activity. On the other hand, in spent fishes from the spawning grounds, while the pituitary and thyroid showed degenerative changes, the adrenal tissue showed hyperplasia and hypertrophy (Unpublished observations from this laboratory). ACTH, cortisone and epinephrine are known to inhibit triglyceride synthesis in the adipose tissue and stimulate the release of FFA. Mashburn et al. (1960) and Rizack (1961) have reported that the activities of certain lipases in the fat depot are increased on incubation with epinephrine. Adipose tissue contains hormones sensitive lipase, as well as an enzymic system for inactivating and reactivating it (Vaughan, 1962). It may be suggested that the hormonal changes taking place during the migratory ascent may be playing an important role in the mobilization of fat from the storage places and its utilization during the period of fasting migrations in fishes also.