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Comparative evaluation of body composition of hilsa, *Tenualosa ilisha* (Hamilton, 1822) in different size groups with special reference to fatty acid, in Hooghly estuarine system, West Bengal, India

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ABSTRACT

Hilsa, *Tenualosa ilish*a (Hamilton) is one of the important commercial fish species in Hooghly estuarine system of West Bengal. Four different size groups of hilsa were collected from two stations in Hooghly estuarine system and analysed for proximate body composition during pre-monsoon and monsoon seasons. Values were compared in pre-monsoon and monsoon periods in the upstream and downstream fishes. Protein content varied from 11.0 to 59.9%, lipid content from 1.32% to 20.85% and carbohydrate content varied from 1.54 to 7.18% in different weight groups of fishes. Saturated fatty acid content ranged from 49.9% to 55.8% and unsaturated fatty acid content ranged between 43.75 and 49.45% during the pre-monsoon period in different weight groups of upstream fishes. ω3 / ω6 ratio varied from 2.5 to 7.0 in different weight groups and ω6 / ω3 varied from 0.14 to 0.40. Unsaturated fatty acid / saturated fatty acid (UFA / SFA) and polyunsaturated fatty acid / saturated fatty acid (PUFA / SFA) were also analysed.

Keywords: Body composition, Hilsa, Hooghly estuarine system, Saturated fatty acids, Unsaturated fatty acids

Introduction

The Hooghly estuarine system along the Indian coast of Bay of Bengal is one of the largest and most productive estuaries in India. It is located in West Bengal between latitude 21º31´ N and 23º30´ N and longitude 87º45´ E and 88º45´E. The total length of the tidal Hooghly estuary is about 295 km. Hilsa (*Tenualosa ilisha*, Clupeidae) forms commercially the most important fishery of the Hooghly estuarine system and the fishery is seasonal in nature (Mitra *et al.*, 1997). According to Day (1889), hilsa is distributed in India along the coastal waters and they ascend the large rivers to breed. In the estuarine areas like the Hooghly, the two main seasons of this fishery are monsoon (July-August to mid October) and winter (mid November to January), when the fish ascends up the river for spawning. Being a migratory fish, it covers a long distance from downstream coastal regions to upstream. Spawning activity appears to be synchronised with the full moon and new moon phases (Miah *et al.*, 1999). According to Brian (1984), migratory fish mobilises proteins before lipids but at the time of spawning migrations, lipid stores are mobilised first and used as a major source of energy. Very little information is available on the biochemical changes in the upstream and downstream hilsa of different size groups in relation to varying seasons. In this paper, an attempt has also been made to correlate body weights and biochemical composition with special reference to fatty acids in downstream and upstream hilsa of the Hooghly estuarine system during pre-monsoon and monsoon periods. The fatty acid profile of lipids also reflect the availability of fatty acids in aquatic food chains (Sargent *et al.,* 2002).

Fish is an excellent source of protein in human diet. Hilsa fish is liked by most of the fish eaters due to its taste and flavour. Biochemical composition is a good indicator of the physiological condition of fish (Ali *et al.*, 2005). It varies within and between species, size, sexual condition, feeding season and physical activity. Protein content tends to vary a little in healthy fish (Weatherly and Gills, 1987). The knowledge of proximate composition of fish is of paramount importance to evaluate its nutritional value and physiological conditions (Chandrashekhar *et al.,* 2004). Currently, all over the world, sea food is encouraged because of its beneficial effects to fight diseases and for maintenance of good health (Carroll and Woodward, 1989). Of all the polyunsaturated fatty acids (PUFA) of marine lipids, eicosapentanoic acid (EPA) ($20:5\omega_3$) and docosahexanoic acid (DHA) ($22:6\omega_3$) have received considerable attention because of their role in various biological activities determining health and disease (Kamp, 1993). In the present study, an attempt has been made to evaluate the body composition of hilsa from the Hooghly estuarine system, with special reference to fatty acid content.

Materials and methods

Along a 126 km stretch of Hooghly River, two sampling stations were selected for investigations. These were the downstream station; station I (Kakdwip, South 24 Parganas) and the upstream station; station II (Tribeny, Hooghly). Sampling was done during March, 2010 to September, 2010. Fishes were collected fortnightly, directly from fishermen in the fishing spot and transported to the laboratory in iced condition. Four weight groups of fishes were collected *viz*., juvenile (10 g+), small (200 g+), medium (500g+) and large (800 g+). After removing fins, scales and viscera, fish tissue were homogenised and analysed for proximate composition. Muscle moisture content was determined following the method of Degani *et al*. (1988). Crude protein was determined by the micro-Kjeldahl method (AOAC, 1990). Ash and crude fiber were determined using standard procedures as described in AOAC (1990). The total lipids were extracted from the fish samples following Bligh and Dyer (1959) using methanol-chloroform (2:1, v/v), methanol-chloroform-water (2:1:0.8, v/v/v), and then again with the first solvent system. Preparation of methyl esters of fatty acids was done following the method of Christie (1982). Purification of fatty acid methyl esters was done by thin layer chromatography following the process of Mangold (1969) and Mishra *et al.* (1984). Gas liquid chromatography (GLC) of fatty acid methyl esters was done on a Chemitto 1000 instrument, equipped with flame ionization detector (FID). Quantification was done using Specific Clarity Lite Software. Identification of fatty acid was carried out by comparing their retention times with those of standards, chromatographed under identical operational conditions of GLC. Nitrogen free extract (NFE or Carbohydrate equivalent) was calculated by subtracting the moisture, crude protein, crude lipid, ash and fibre from 100%. NFE%= 100- (moisture+ crude protein+ crude lipid+ ash+ crude fiber). Calorific value or gross energy contents in the muscle was determined using an adiabatic bomb colorimeter (Gallenkamp) using standard protocols.

Statistical analysis of the results was done following the method of Snedecor and Cochran (1967). The correlation analysis was performed to detect differences ($p<0.05$) in proximate body composition between upstream and downstream fish groups.

Results and discussion

Moisture content was found to be above 50% in all samples anlysed (Table 1 and 2). Carbohydrates are the principal and immediate energy source for organisms. Carbohydrate concentration did not vary much in all the samples examined (Table 1 and 2). Liver and muscle glycogen constitute energy rich compounds that can be mobilised and used for locomotion. The results indicate that hilsa does not primarily rely upon carbohydrates during migration. This supports the view of Brian (1984) that carbohydrate concentration does not vary much during migration. The concentration of total protein is found to be higher in juveniles and it is about 59.9%. In small to large sized fish groups from both upstream and downstream stations, crude protein values varied from 11.0 – 21.23% (Table 1 and 2). Protein value in small fish groups (17.79 %) was found to be higher than that in the medium (11.0) and large fishes (13.23 %) during the monsoon period (Table 2). In general, except in juvenile's, protein values did not vary much .

Table 1. Biochemical composition of hilsa from upstream station of Hooghly estuarine system during pre-monsoon period

Body constituents	Juvenile (10 g+)	Small (200 g+)	Medium (500 g+)	Large (800 g+)
Moisture (%)	16.09 (0.12)	74.36 (1.29)	57.4 (0.66)	68.04 (1.32)
Lipid (%)	1.32 (0.28)	1.99 (0.30)	2.48 (0.25)	7.82 (0.07)
Crude protein (%)	59.9 (0.22)	15.39 (0.57)	21.23 (0.23)	15.58 (0.33)
Carbohydrate (%)	1.54 (0.03)	2.60 (0.21)	7.18 (0.06)	3.14 (0.07)
Cal. value (Cal. g^{-1})	456.0 (3.61)	143.83 (2.41)	260.0 (3.23)	196.81 (0.79)

Standard deviations are given within parenthesis

Table 2. Biochemical composition of hilsa in downstream and upstream stations of Hooghly estuarine system during monsoon period

Body constituents	Downstream (Station 1)		Upstream (Station 2)
	Small (200 g+)	Medium (500 g+)	Large (800 g+)
Moisture (%)	57.66 (0.41)	45.77 (0.42)	58.70 (0.17)
Lipid (%)	14.45 (0.14)	20.85 (0.17)	13.71 (0.14)
Crude protein (%)	17.79 (0.09)	11.0 (0.33)	13.23 (0.15)
Carbohydrate (%)	3.45 (0.07)	5.34 (0.33)	3.41 (0.16)
Cal. value (Cal. g^{-1})	291.37 (1.64)	427.32 (1.55)	304.98 (0.29)

Standard deviations are given within parenthesis

In addition to protein, lipids play diverse role in nutrition and health. During migration when they ascend upstream the river for breeding , due to less intake of food, hilsa utilises their body lipids as a source of energy. Lipid content is affected by season, maturity stages as well as the habitat of the fishes. Small fishes from downstream station had 14.40% lipid and 1.35% lipid during monsoon period (Table 1 and 2). Medium size group fishes from downstream had 21.0% lipid during monsoon whereas upstream fish of similar size group had only 2 - 3% lipid during pre-monsoon period. Large sized fishes contained 14% lipids during monsoon, but fishes of same group had 7-8% lipid during pre-monsoon period. The increase in lipid content during monsoon indicates that the fishes are in mature condition. Purely marine fish species generally have higher level of lipids as in case of pomfret with about 32.31% lipid in body muscles (Huang, 2010). Hilsa, being a migratory fish, lipid content varies widely which is evident from the present results. Previous reports shows that the fat content in hilsa of medium size group is 16.35% (Mohanty *et al.*, 2011) which is a little less from the present values.

The present study reveals that saturated fatty acid (SFA) values are higher than unsaturated fatty acid (UFA) values (Table 3). Small upstream fish groups and large fishes were found to have PUFA values ranging between 10.1 to 17.5%. PUFA values ranged between 8.15 to 26.8% in the present study. Small and medium fish groups from downstream station had 8.15 to 12.1 % PUFA. SFA was maximum (49.9-57.32%) compared to MUFA and PUFA in all groups of fishes from both upstream and downstream stations (Table 4). Hilsa might be unable to use SFA as an energy source efficiently when compared to MUFA. During monsoon period, large-sized group of upstream hilsa had about 4.5% EPA (20:5 ω_3) and about 0.8% DHA (22:6 ω_3) (Table 5). During the same period, downstream medium sized fish group contained 3.6% EPA and 0.9% DHA. Small fish group of the downstream station had 6% EPA and 0.6% DHA. On the other hand, during pre-monsoon period, upstream juveniles contained 5.8% EPA and 5.6% DHA. Except juveniles, other fish groups of pre-monsoon season had about 6.6%, 6.3%, 7.2% EPA and 2.6%, 3.9% and 1.8% DHA respectively. Ratio of DHA/EPA was found to be maximum (0.96) in juvenile groups (Table 3). ω_3/ω_6 ratio was higher (3.2-7.0) in upstream fishes during pre-monsoon period as compared to that of monsoon period (2.5-3). Large upstream fish group showed ω_3/ω_6 ratio of 2.66 and downstream small and medium fish groups showed ratios of 3.0 and 2.5 respectively (Table 3). In the present study, the higher concentration of ω_3 PUFA compared to ω_6 PUFA indicates the preferential incorporation of ω_3 PUFA. Ackman (1994) opined that freshwater and tropical oceanic fish commonly contain large percentages of ω_6 PUFA; especially arachidonic acid (20:4 ω_6, AA). This slightly differs with the present findings that hilsa has higher percentages of ω_3 PUFA. Among ω_6 PUFA, arachidonic acid content was found highest. Both 18:2 ω_6 (linoleic acid) and 20:4 ω_6 (AA) have a significant biological role; especially with respect to eicosanoids derived from 20:4 ω_6 that are essential for reproduction

Table 3. Percentage of fatty acids in different weight groups of hilsa collected from upstream and downstream stations of Hooghly estuarine system during pre-monsoon (March - May, 2010) and monsoon period (July- Serptember, 2010)

Constituents	Pre-monsoon period				Monsoon Period		
	Upstream (Station 1)				Upstream (Station 1)	Downstream (Station 2)	
	Juvenile	Small	Medium	Large	Large	Small	Medium
Lipid (%)	1.32	1.99	2.48	7.82	13.71	14.45	20.85
Fatty acid (%)							
SFA	49.9	55.8	50.74	49.92	53.01	52.7	57.32
UFA	49.6	43.75	48.4	49.45	46.8	46.8	42.88
Mono UFA	22.8	29.05	30.9	34.05	36.7	34.7	34.73
PUFA	26.8	14.7	17.5	12.7	10.1	12.1	8.15
ω_5	3.7	0.3	0.3	0.1	0.2	0.1	0.1
ω_3	17.6	12.6	14.4	12.3	7.2	9.0	5.75
ω_6	5.5	1.8	2.8	3.0	2.7	3.0	2.3
ω_3/ω_6	3.2	7.0	5.14	4.1	2.66	3.0	2.5
ω_6/ω_3	0.31	0.14	0.19	0.24	0.38	0.33	0.40
UFA/SFA	0.99	0.78	0.95	0.99	0.883	0.658	0.748
PUFA/SFA	0.54	0.26	0.34	0.25	0.19	0.23	0.14
DHA / EPA[1]	0.96	0.39	0.62	0.25	0.18	0.15	0.17

1 = 22: 6 ω_3 / 20: 5 ω_3

Table 4. Important MUFA and SFA in hilsa (mean values are given within parenthesis)

	Pre-monsoon				Monsoon		
	Upstream (Station 1)				Downstream (Station 1)		Upstream (Satation 2)
	Juveniles	Small	Medium	Large	Small	Medium	Large
MUFA	16:1 (11.8)	16:1 (8.8)	16:1 (11.8)		16:1 (12.5)	16:1 (11.7)	16:1 (13.0)
		17:1 (3.2)	17:1 (1.6)	17:1 (13.2)	17:1 (1.3)	17:1 (1.1)	17:1 (0.8)
	18:1ω9 (9.5)	18:1ω9 (16.9)	18:1ω9 (17.1)	18:1ω9 (18.4)	18:1ω9 (20.6)	18:1ω9 (21.8)	18:1ω9 (22.6)
SFA	14:0 (4.6)	14:0 (9.9)	14:0 (11.2)	14:0 (10.4)	14:0 (9.2)	14:0 (10.4)	14:0 (11.1)
	15:0 (1.4)						
		17:0 (1.2)	17:0 (1.0)	17:0 (1.1)	17:0 (0.8)	17:0 (0.8)	17:0 (0.7)
	18:0 (11.1)	18:0 (5.8)	18:0 (6.8)	18:0 (6.0)	18:0 (7.5)	18:0 (6.7)	18:0 (6.7)
					22:0 (0.6)	22:0 (0.5)	22:0 (0.6)
	24:0 (2.0)						

Table 5. Important PUFA in hilsa (mean values are given within parenthesis)

Pre-monsoon				Monsoon		
	Upstream (Station 1)			Downstream (Station 1)		Upstream (Satation 2)
Juveniles	Small	Medium	Large	Small	Medium	Large
16:2ω5 (3.7)						
18:2ω6 (3.5)	18:2ω6 (0.5)	18:2ω6 (0.7)	18:2ω6 (0.7)	18:2ω6 (0.5)	18:2ω6 (0.5)	
18:3ω3 (2.4)	18:3ω3 (0.8)	18:3ω3 (0.7)	18:3ω3 (0.7)	18:3ω3 (0.5)	18:3ω3 (0.5)	
20:4ω6 (1.2)	20:4ω6 (0.9)	20:4ω6 (1.5)	20:4ω6 (1.6)	20:4ω6 (1.8)	20:4ω6 (1.3)	20:4ω6 (1.7)
20:4ω3 (2.6)	20:4ω3 (1.5)	20:4ω3 (2.2)	20:4ω3 (1.4)	20:4ω3 (0.7)	20:4ω3 (0.5)	20:4ω3 (0.7)
				22:4ω6 (0.5)		
20:5ω3 (5.8)	20:5ω3 (6.6)	20:5ω3 (6.3)	20:5ω3 (7.2)	20:5ω3 (6.0)	20:5ω3 (3.6)	20:5ω3 (4.5)
22:5ω3 (0.8)	22:5ω3 (0.7)	22:5ω3 (1.1)	22:5ω3 (1.1)	22:5ω3 (0.8)	22:5ω3 (0.5)	22:5ω3 (0.8)
22:6ω3 (5.6)	22:6ω3 (2.6)	22:6ω3 (3.9)	22:6ω3 (1.8)	22:6ω3 (0.9)	22:6ω3 (0.6)	22:6ω3 (0.8)

and cellular signal transduction in fish (Bell and Sargent, 2003). Elevation of 20:4 ω_6 can improve growth and survival (Bessonart *et al.*, 1999). ω_6/ω_3 ratio ranged from 0.14 to 0.31 in different sized group of upstream fishes during pre-monsoon period. During the monsoon period this ratio showed much higher value *i.e.*, from 0.33 to 0.40. The ratios of ω_6/ω_3 in both upstream and downstream hilsa fishes found in the study were far less than the optimum value (4.0 maximum) recommended for human health by the UK Department of Health.

Regarding moisture content in hilsa, a negative correlation ($p<0.05$) was noticed in the small sized fish group between upstream and downstream stations during pre-monsoon and monsoon periods. Negative correlation was also noticed between medium and large size groups in the upstream station during pre-monsoon period and positive correlation was found between other groups. With increasing body weight, moisture content also increases. The percentage of water is a good indicator of the relative contents of energy, proteins and lipids. According to Dempson *et al.* (2004), lower the percentage of water, greater the lipid and protein contents and higher the energy content of the fish. In large sized fish groups, with increasing body weight moisture content also increases. Here inverse relationship was found between lipid and moisture content of monsoonal fishes. But in all other cases such generalised relation is not always observed. Protein value in small fish groups (17.79 %) is little higher than the larger ones (13.23 %) during the monsoon period (Table 2). Except in juveniles, protein values are not found to vary much. Present observation differs from the reports of CIFRI (2009-2010) indicating higher protein level (13.23%) in large sized hilsa. The adult fishes have least protein content of 11-13.23% during monsoon period (Table 2) which indicates that they consume less food or it may be due to starvation that their protein content did not increase (Lucas and Baras, 2001). Chandrashekhar *et al.* (2004) opined that hilsa is mostly a plankton feeder. Significant difference ($p<0.05$) in the protein values were observed in large fish groups upstream and between medium sized group of upstream and downstream fishes during pre-monsoon and monsoon period. When animals start migration their fat content is found to be higher (Krapu *et al*., 1985). In Salmon, protein: fat ratio varies in such a way that these fishes utilise more fat than protein for better growth and reproduction (Hillestad and Johnsen, 1994). In tuna and mackerel also fat replaces protein in some cases for energy utilisations (Mannan *et al.*, 1961). During the present study period, low protein: fat ratios were found in all the monsoonal hilsa which indicate their preparation for spawning migration.

In addition to protein, lipids play an important and diverse role in nutrition and health. During migration when they ascend up the river for breeding purposes, due to less intake of food, hilsa utilises their body lipids for providing necessary energy to the body. During pre-monsoon period lipid content was found to be high (about 7.82%) in the large fish group in comparison to the other upstream fish groups, which indicates that they have grown and matured in the upstream region. High lipid content in downstream small and medium sized fish groups during monsoon period indicates they are ready for migration. Lipid content is affected by season, maturity as well as habitat of the fishes. Present observation reveals that young hilsa have high nutritive value in terms of protein and fat content. Higher level of SFA than of MUFA indicaes that hilsa may be unable to use SFA as an energy source efficiently when compared to MUFA, and therefore SFA tends to accumulate in their tissues. Several previous studies claimed MUFA as a good substrate for β-oxidation in fish (Henderson and Sargent, 1985; Sidell *et al*., 1995; Stubhaug *et al*., 2005). According to Daniela (2005), SFA and MUFA are storage lipids preferentially used as energy sources. SFA was maximum (49.9 - 57.32%) compared to MUFA and PUFA in all groups of fishes in both upstream and downstream (Table 4). The higher level of PUFA was primarily due to higher levels of 18:2$\omega_{6,}$ 18:3$\omega_{6,}$ 20:4ω_6, 20:4$\omega_{3,}$ 20:5ω_3 and 22:6ω_6 in all groups of fishes in both upstream and downstream stations during pre-monsoon and post-monsoon periods (Table 5). In juveniles, 16:2ω_5 was found abundantly. MUFA in fish muscles were 16:1, 16:2, 18:1ω_5 and 18:2ω_6 in different groups of fishes and prominent SFA were 14:0, 15:0, 16:0, 18:0 and that in juveniles were 24:0 in addition to the above (Table 4). EPA and DHA cannot be synthesised *de novo* in the body and therefore, are derived from diet or dietary supplements. Reduced triglyceride level is the most consistent hypolipidemic action of EPA and DHA. Thus, hilsa is a good diet to supplement EPA and DHA deficiency. Present study reveals that DHA is less in downstream fishes. In upstream fishes, DHA was found to decrease with increasing body weight. Ratio of DHA/EPA was found maximum (0.96) in juveniles (Table 3).

The ratio of total ω_6 PUFA to ω_3 PUFA in fish muscle of all groups reflected those of the diets. In the present study, the higher concentration of ω_3 PUFA as compared to ω_6 PUFA indicates the preferential incorporation of ω_3 PUFA. Ackman (1994) opined that freshwater and tropical oceanic fish commonly contain large percentages of ω_6 PUFA; especially arachidonic acid (20:4 $\omega_{6,}$ AA) which differs with the present findings that hilsa contains higher percentages of ω_3 PUFA than ω_6 though among ω_6 PUFA, arachidonic acid content is highest. Both 18:2 ω_6 (linoleic acid) and 20:4 ω_6 (AA) have significant biological role; especially with respect to eicosanoids derived from 20:4 ω_6 that are physiologically active in fish and are essential for reproduction and cellular signal transduction in fish (Bell and Sargent, 2003). Elevation of 20:4 ω_6 can improve growth and survival (Bessonart *et al.*, 1999). ω_3 is a very important fatty acid having various beneficial effects like, anti-inflammatory, antithrombotic, hypolipodermic and vasodialatory properties in human health. On the other hand ω_6 fatty acids promote platelet aggregration, vasoconstriction *etc*. The ω_6 /ω_3 fatty acid values of 1-2:1 is reported to be optimum for human health (Simopoulos, 1999). The value of 0.14-0.40:1 recorded in hilsa, suggests that it is a highly beneficial food source to supply ω_3 to the human body. Moreover, less ω_6 and high ω_3 contents were recorded in upstream pre-monsoon fishes. Therefore, when these fishes are suggested as a means of improving human health, both lipid and PUFA contents in their body muscle need to be considered.

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