

Original Research Article

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## Changes in the Biochemical and Mineral Composition of Hilsa Shad, *Tenulosa ilisha* (Hamilton, 1822) during Upstream Spawning Migration

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### ABSTRACT

A study was conducted to examine the changes in biochemical composition consisting moisture, protein, lipid, carbohydrate, ash content and mineral composition of Indian shad, hilsa (*T. ilisha*) during upstream migration for spawning from off-shore of the Bay of Bengal to the Bhagirathi-Hooghly zones of the Ganga river system in India. Adult hilsa fish were collected from seawater (SW), freshwater 1 (FW1) and freshwater 2 (FW2) locations, where the salinity level was 26-28‰, 1-5‰ and 0-0.04‰ respectively. The moisture and carbohydrate varied significantly among three locations, but not the protein and ash contents. The moisture content of hilsa collected from different habitats ranged between 62.40% and 68.71%, whereas, the protein, lipid, carbohydrate and ash percentages were 15.22%, 12.61%, 0.84%, 2.59% and 15.59%, 14.35%, 3.85%, 3.78% in seawater and freshwater respectively. The minerals like sodium, potassium and calcium were highest in SW hilsa compared to FW hilsa. However, iron content was highest in FW hilsa compared to SW hilsa. The results indicate that the migration influences the nutritive value of hilsa, as the lipid, sodium, potassium and calcium levels significantly reduced during upstream river migration. It further, point out that the lipid and carbohydrate mobilized as the energy source to support long migration and gonadal development. The ionic profile of hilsa muscle showed location effects, and the values declined while the fish migrated through freshwater indicates that the energy driven metabolic processes might govern the acclimation of upstream migration and spawning of *T. ilisha*.

#### Keywords

Hilsa, Biochemical composition, Ionic composition, Upstream migration, Seawater, Freshwater

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### Introduction

Indian shad, hilsa is recognized as one of the most delicious, commercial fish of the Indo-Pacific region, which belongs to the subfamily Alosinae of Family Clupeidae. The hilsa shad is a long distance anadromous fish

which migrates from its marine environment to the freshwater rivers for spawning. From offshore water of the Bay of Bengal, the fish ascend into the Ganga River twice a year, during February to March, and September to October (Ahasan *et al.*, 2014). It has a broad range of geographical distribution and found

along marine, estuarine and riverine waters of Persian Gulf, Red Sea, Arabian Sea, Bay of Bengal, Vietnam Sea and China Sea. Indian rivers, namely the Hooghly Bhagirathi stretch of the Ganga, Godavari, Narmada, Tapti and other coastal rivers bestowed with hilsa fishery. The significant portion of hilsa (about 90%) caught by Bangladesh, India and Myanmar (Bhaumik, 2013). The hilsa is a highly prized food fish accounting 15-20% of the total fish landings of the Hooghly estuary (Mohanty *et al.*, 2011). The nutritional importance of fish consumption to a great extent associated with its protein, unsaturated essential fatty acids, minerals and vitamins (Sidhu, 2003).

Fish and seafood play a significant role in human nutrition and health, which provide the balance of proteins, lipids, vitamins, minerals and have a relatively low caloric value than other muscle foods. The flesh of hilsa is an excellent source of proteins in the human diet and preferred by most of the fish eaters due to its pleasant taste and smell. The biochemical composition is a good indicator of the physiological condition of fish (Ali *et al.*, 2005).

The nutritional composition of fish varies greatly from species to species and within the species, depending on age, feed intake, physical activity, sex and sexual changes connected with spawning, environment or geographical localities and season. Energy resources partition between an animal's metabolic activity, growth performance and reproduction. The nutritional status and consequently muscle composition is directly affected by the reproductive activity. Principal constituents of fish is 16-21% protein, 0.2-25% fat, 1.2-1.5% carbohydrate and 66-81% water (Love, 1970). The biochemical composition varies greatly from species to species and also from individual to individual depending on age, sex,

environment and season (Huss, 1988). During somatic growth, the protein, lipid, and ash typically accumulate while protein and lipid deplete during gonadal growth (Tanasschuk, 1989). Mostly anadromous fish accumulate a large amount of energy reserves prior to begin migration for spawning.

Although there is wealth of information on the biochemical composition of hilsa shad from different habitats (Rao *et al.*, 2012; Begaum *et al.*, 2016; Ganguly *et al.*, 2017), changes in proximate and mineral composition in hilsa during upriver spawning migration from off-shore Bay of Bengal into Hooghly-Bhagirathi stretches of the Ganga river is limited. The present study was taken up with an objective to analyse the changes in the biochemical and mineral composition of hilsa shad during its anadromous migration from the Bay of Bengal to the Bhagirathi-Hooghly stretches of the Ganga River.

## **Materials and Methods**

### **Sample collection, preservation and preparation**

The experimental animals of the study were hilsa adults (*Tenualosa ilisha*) with an average weight ranging from 225 gm to 470 gm. The 15 fish samples were collected from the Digha (SW; 26 to 28‰), Nischintopur (FW1; 1 to 5‰) and Shyamnagar (FW2; 0 to 0.04‰) along Bhagirathi-Hooghly stretch of the Ganga river system in the West Bengal. The collected live fish samples were washed properly with deionised water to remove all dirt's, slime, and length and weight of the fish were recorded.

The fish were cut into fillets, packed in plastic bags and transported in dry ice to the Central Institute of Fisheries Education, Kolkata centre, West Bengal. Fifteen fish used for taking samples for all biochemical analysis.

### **Physicochemical parameters of water**

The physico-chemical parameters such as, temperature, salinity, alkalinity, total suspended solids (TSS) and total dissolved solids (TDS) estimated using standard methods (APHA, 2017).

### **Proximate analysis**

For proximate analysis, all the dissected fishes from different salinities weighed and kept in pre weighed Petri plates. Moisture, protein, lipid, carbohydrate and ash were determined as per standard methods (AOAC, 2006). Moisture (%) was calculated after drying the different sampled fish were dried in hot air oven at  $100^{\circ}\text{C} \pm 2$  till a constant weight. After complete drying, the fish of different salinities were ground into the fine powder with a pestle and mortar. Crude protein content ( $\text{N}\% \times 6.25$ ) was estimated after acid digestion using semi-automatic nitrogen analyzer (2200 Kjeltac auto distillation; Foss Tecator, Hoganas Sweden). Crude lipid was determined by the ether-extraction method in a soxhlet extraction apparatus (Socplus, SCS-08-AS, Pelican equipment, Chennai, India), ash content was determined after burning the dried samples in muffle furnace at  $550^{\circ}\text{C}$  for 6 h and carbohydrate was determined by subtracting the water, protein, fat and ash from 100.

### **Osmolality and minerals**

Water osmolality of FW and SW samples were measured using a vapour pressure osmometer (Model 5600). Concentration of ions such as sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ), calcium ( $\text{Ca}^{++}$ ) ions of water as well as muscle were measured using Eschweiler Combi Blood Electrolyte Analyzer (Diamond Diagnostics-USA). The iron content from digested muscle samples were analyzed by atomic absorption

spectrophotometer (A Analyst 800, Perkin Elmer) according to the manufacturer's instructions.

### **Statistical analysis**

Data collected were subjected to one way analysis of variance (ANOVA) and statistical comparisons between treatments were made by the Tukey honest significant difference (HSD) test using SPSS version 12.0 software for Windows. The significance of observed differences was tested at  $p < 0.05$ .

### **Results and Discussion**

Upstream migrations in lotic systems are energetically demanding, and mostly feeding during long-distance migrations is scarce. As a result, anadromous species rely heavily on energy reserves that they accumulate in the months preceding migration. The degree of utilizing energy reserves is highly variable both among species and populations. The iteroparous species deplete only 35 to 60% of their stored energy compared to 75 to 82% in semelparous species like salmon, shads. Also, migration associated with gonad development and spawning demands much more power. Hilsa being an anadromous and iteroparous fish must face such depletion during spawning migration run and which positively influences central energy reserves, such as, proteins, carbohydrates, and lipids. Moreover, mineral contents may also vary owing to change in the ionic environment during migration. As consequences, nutritional values of hilsa may be increased as reported in the hilsa from Godavari River (Rao *et al.*, 2012) and earns many consumers preference.

It is essential to understand how environmental parameters vary at different locations across the migratory path, which ultimately dictates physiological strategies of fish for acclimating in various saline habitats.

The sampling locations i.e., SW, FW1 and FW2 exhibited remarkable variations in physiochemical parameters such as temperature, salinity, alkalinity, TSS, TDS, Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> and Ca<sup>++</sup> ions concentrations and osmolality (Table 1). As expected, the ion concentrations were highest in the SW compared to the FW1 and FW2. When hilsa migrate from SW to FW environment and vice versa as other anadromous species, they need to adjust ionic and osmotic balance in their internal milieus differently to the salinities of external environment for their survival in hypertonic SW or hypotonic FW.

In the present study, the biochemical composition of *T. ilisha* collected from (SW) and FW1 and FW2 in the Ganga river system; the FW2 was away from saltwater intrusion. The changes in the biochemical composition of whole fish consisting moisture, crude protein, total lipid, total carbohydrates, and ash during anadromous migration from marine to the riverine environment are presented in Figure 1.

The moisture content of hilsa fluctuated from 62.40% to 68.71%; the lowest moisture content was recorded in SW (62.40%), the value increased in the fish migrated to the upstream river at FW1 (64.56%) and FW2, (68.71%). The moisture content was within the range (58.82 to 69.54%) as reported by Majumda and Basu (2009) in hilsa from Bangladesh and 66.90 % from FW environment (Mohanty *et al.*, 2017). The moisture content of fish muscle has an inverse relationship with lipids and proteins (Lone and Matty, 1980). Protein is an indispensable nutrient required for the structure and function of all living organisms. The protein content of the muscle varies widely depending on factors such as feeding habits and availability of food, fasting, and migration, etc. (Sahikhmahmud and Magar, 1957). Muscle protein depleted during periods

of fasting or starvation. The variation in the protein content of hilsa during anadromous migration was not very conspicuous. In the present study, there was no significant difference in protein content, although it was numerically higher in the SW hilsa (15.59%) as compared to FW1 (15.24%) and FW2 (15.22%). In the present study, the muscle protein levels in different saline environments were less than those reported earlier, the range of 16.80 to 20.7% (Kamal *et al.*, 1996; Majumdar and Basu, 2009; Majumdar and Basu, 2010; Hossain *et al.*, 2014; Mohanty *et al.*, 2017). The highest ash content was observed in SW (3.78%) compared to those at FW1 (3.06%) and FW2 (2.59%) respectively. However, the muscle ash content reported in the study was slightly higher than those reported earlier as 1.1% (Mohanty *et al.*, 2017) and 2.27% (Saha and Guha, 1939). Total carbohydrate content was in the range of 0.84 to 3.85%, and the highest value in SW hilsa declined when the fish migrated into FW locations.

The fat contents showed a significant difference ( $p < 0.05$ ) in SW, FW1 and FW2 hilsa during its anadromous migration. The highest fat content in the SW (14.35%) depleted while the hilsa migrated into FW1 (13.53%) and in FW2 (12.61%). The values were higher than the earlier report (11.85%) of hilsa from the Sudarban estuary of West Bengal, India (Pal *et al.*, 2011), whereas the present values were lower than those reported as 17.56% in hilsa from the Bay of Bengal (Kamal *et al.*, 1996). Nath and Banerjee (2012), Rao *et al.*, (2012) and Majumdar and Basu (2009) reported muscle lipid contents of 17.30%, 20.85% and 20.78% in hilsa from brackish water habitat in West Bengal, downstream of Hoogly River and Bangladesh, respectively. The pattern of lipid composition fluctuates and governs by the rate of fat metabolism, maturity stage, environmental temperature, food availability, stress and other

factors (Sikorski *et al.*, 1990). The higher lipid content found in hilsa from the Bay of Bengal could be related to their foods and active feeding behaviour. The biochemical composition of fish is strongly affected by food contents (Henderson and Tocher, 1987; Orban *et al.*, 2007). Hilsa feeds on plankton, mainly by filtering, but also by grubbing on the muddy bottom. The fat content decrease in spawning period is due to mobilization of fat related to gametogenesis (Sharer, 1994).

Our results suggest that fish accumulate fat in the marine environment before the initiation of spawning and then proceed upwards into the Bhagirathi-Hooghly stretch of the Ganga river system. Hilsa accumulate energy reserves during their growth phase in the form of lipids, mainly as triglycerides which are catabolized to provide the energy which is necessary for anadromous migration and gametogenesis. Jonsson *et al.*, (1997) reported a decrease in lipid content during the upward migration of Atlantic salmon. Body lipid decreased by 30 to 40% during the period of re-entry of Arctic charr to FW from SW, and the female fish lost 80% of their body lipids during spawning (Josrgensen *et al.*, 1997). The migration of European eels is heavily dependent on the fats stored during their growth phase (Boetius and Boetius, 1985).

Minerals are essential for growth, bone mineralization, reproduction, and energy metabolism in all living organisms. The significant portion of minerals in the fish body concentrated in muscle, scale, and vertebrae (Lall, 2002). The prominence of each mineral element in body tissue is closely related to its functional role. The macro-minerals which include calcium, phosphorous, magnesium, sodium, chloride and potassium occur in the body at a concentration ranging from 0.1 to 2.0% of fish mass (Lall, 1995). Earlier evidence support that the hilsa flesh is a rich source of

various essential minerals. The present study showed the  $K^+$  was the most abundant elements in muscle followed by  $Na^+$ ,  $Ca^{++}$  and Fe content (Table 2).

The muscle  $K^+$  levels (601-1203 mg/100g) were slightly lower than those of 1390 mg/100g reported for *Pseudotolithus elongates* muscle (Njinkoue *et al.*, 2016); however, it was similar to 613 mg/100g and 573 mg/ 100g in hilsa muscle collected from the Bay of Bengal (Hossain *et al.*, 2014; Rao *et al.*, 2012).  $Ca^{++}$  is the essential nutrients for growth and significant constituent of the structural components of skeletal tissues. The  $Ca^{++}$  level in the muscle ranged from 120 to 168 mg/100g) and was similar (155-204 mg/100g) to the hilsa of Bay of Bengal (Hossain *et al.*, 2014).

The muscle  $Na^+$  content ranged between 82.26 and 199.6 mg/100g in SW and FW environments, which is similar to the marine hilsa (183 mg/100g) from the Bay of Bengal and Godavari hilsa (83 mg/100g) as reported by Rao *et al.*, (2012). Iron plays a critical role within cells assisting in haemoglobin synthesis, oxygen utilisation, enzymatic systems, especially for neural development and overall cell function all over the body. The iron (Fe) level in the muscle varied between 1.46 and 2.67 mg/100g during migration, the level in FW was higher compared to the hilsa intercepted from SW locations. The present level of muscle iron was similar to the average iron value reported in different sizes of hilsa of the Ganga River (Ganguly *et al.*, 2017) and Bay of Bengal, however it was less than the value reported from the Arabian Gulf (Hossian *et al.*, 2014). The concentration of minerals and trace element levels are known to vary in fish depending on various factors such as their feeding behaviour, environment, ecosystem and migration (Andres *et al.*, 2000).

**Table.1** Water quality parameters of different habitats

Parameters	SW	FW1	FW2
Temperature (°C)	25.5-25.50	23-23.50	22-23.00
Ph	8.15	7.75	7.65
Salinity (‰)	26.00-28.00	1.00-5.00	0.00-0.04
Osmolality (mmol/kg)	810.50 <sup>a</sup> ±4.50	76.00 <sup>b</sup> ±1.00	0.00 <sup>c</sup> ±0.00
TSS (mg/L)	163.15 <sup>b</sup> ±6.29	406.16 <sup>a</sup> ±9.45	74.13 <sup>c</sup> ±5.15
TDS (mg/L)	34198.68 <sup>a</sup> ±534.08	3692.88 <sup>b</sup> ±145.97	59.16 <sup>c</sup> ±5.05
Alkalinity (mg/L)	134.33 <sup>a</sup> ±3.28	142.0 <sup>a</sup> ±4.04	104.33 <sup>b</sup> ±2.18
Ca <sup>++</sup> (mg/100mL)	27.91 <sup>a</sup> ± 1.14	5.75 <sup>b</sup> ± 0.89	3.48 <sup>c</sup> ± 0.53
K <sup>+</sup> (mg/100mL)	33.21 <sup>a</sup> ± 0.13	3.94 <sup>b</sup> ± 0.23	0.76 <sup>c</sup> ± 0.05
Na <sup>+</sup> (mg/100ML)	846.81 <sup>a</sup> ± 14.18	110.30 <sup>b</sup> ± 3.20	17.23 <sup>c</sup> ± 1.77
Cl <sup>-</sup> (mg/100ML)	1169.85 <sup>a</sup> ± 25.18	191.43 <sup>b</sup> ± 5.02	40.76 <sup>c</sup> ± 3.16

Water physico-chemical parameters at different locations SW, FW1 and FW2. Mean values bearing different superscripts under each row varied significant (p<0.05). Data expressed as Mean ± SE, (n=6)

**Table.2** Mineral composition of hilsa muscle from different habitats (dry weight basis; mg/100 g)

Muscle sample	Iron	Calcium	Sodium	Potassium
SW	1.46 <sup>c</sup> ±0.11	1680 <sup>a</sup> ±9.86	199.60 <sup>a</sup> ±8.79	1203.86 <sup>a</sup> ±12.04
FW1	2.28 <sup>b</sup> ±0.04	140.33 <sup>b</sup> ±2.90	106.20 <sup>b</sup> ±7.37	714.60 <sup>b</sup> ±7.96
FW2	2.67 <sup>a</sup> ±0.10	120.00 <sup>c</sup> ±4.35	82.26 <sup>c</sup> ±3.3	601.00 <sup>c</sup> ±10.57

Values are expressed as Mean ± SE, (n =6). Values in the same column with different superscript letters are significantly different (P<0.05). SW (26 to 28‰), FW1 (1 to 5‰) and FW2 (0 to 0.04‰)

**Fig.1** Changes in biochemical composition of whole body of *Tenualosa ilisha* adults during upstream migration. Values are expressed as Mean ± SE, (n=15). The bar bearing different superscript differ significantly (p<0.05). SW (26 to 28‰), BW (1 to 5‰) and FW (0 to 0.04‰)

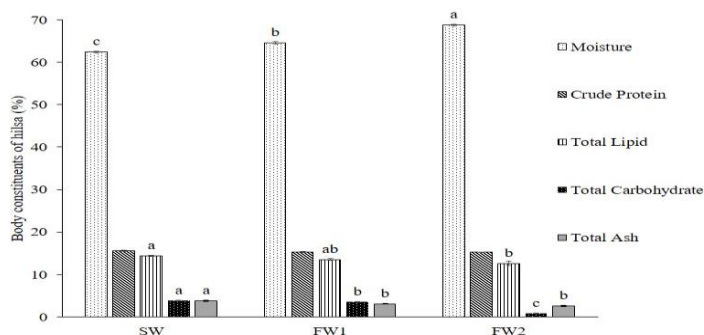


Figure 1

In conclusion, supply of energy is essential for osmoregulatory acclimation in the changing environment and spawning during migration in fish. As the lipid is the principal substrate of energy, the depletion and mobilization of fat was more prominent and might be linked with both osmoregulation and spawning in the fish. The present study reveals that lipid and ionic levels of muscles depleted in hilsa while migrated from the off-shore Bay of Bengal into freshwater stretches of the river Ganga supporting the enzyme-mediated metabolic processes might govern the acclimation of upstream migration and spawning of *T. ilisha*.

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