1. Introduction
The unrevealed silence of marine world is blessed with diversified aquatic wealth including edible fishes. 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 21°31´N and 23°30´N and longitude 87°45´E and 88°45´E. The total length of the tidal Hooghly estuary is about 295 km. FAO species survey reports that 475 species of fishes under 138 families existed in Bay of Bengal (Huq et al., 1999) most of which are still unutilized or poorly known for their nutritional quality. In addition to energy supplier, marine fishes are consumed as a source of polyunsaturated fatty acid (PUFA) and vitamins (Stanby, 1973) though the composition of fatty acid in the edible part of fish is affected by many factors, such as species, sex, sexual maturity, size, place of capture, water temperature, feeding and season (Botta et al., 1986; Armstrong et al., 1991).

Fish is quite different from the other animal food sources. Particularly compared to red meat fish provides high protein with polyunsaturated fatty acids and the fish flesh is easily digestible because it contains long muscle fiber (Kandemir and Polat, 2007). The value of fish as a source of long chain ω3 fatty acid primarily depends on the lipid content in the muscle and the region from which the fishes are harvested. Usually, the fatty fishes caught from colder region contain the highest amounts of lipids (Sikorski and Kolakowska, 2003). Mitra et al. (1977) had reported that lipid content of fish varies according to the season and is related to their breeding cycles.

The main characteristic difference in freshwater fish is the higher levels of C16 and C18 acids and the lower levels of C20, and C22 acids when compared to marine fish, and these differences are mainly due to dietary fat (Ackman, 1967; Nair and Gopakumar, 1978). Ackman et al. (2002) suggested the liver of lean fish shows a much higher level of lipid classes than those of muscles. It was also suggested that the liver is the chief site of lipid synthesis as well as of storage but usually the gut contents are discarded during processing of fish as food. Thus the muscle lipid becomes the chief source of fish lipid for consumption by human.

A major nutrition-related problem in India is chronic undernutrition associated with low fat intake. Low level nutrition starts even at the time of conception (because of maternal undernutrition) and a large number of infants are born with low birth weight (Majumder et al., 2013). Country like India where a major percentage of people belongs below poverty level consumes fish particularly marine fish to combat the situation of nutritional deficiency but still
the assessment of fat quality particularly from the aspect of lipid and fatty acid is not done so far. This study focuses on the fatty acid composition of four commercially cheap marine fish species found in Bay of Bengal by Thin layer chromatography (TLC) and Gas Liquid Chromatography (GLC). The available data can be compared with the other edible marine fishes to obtain the nutritional quality of those fishes under study from the aspect of lipid and fatty acid composition. The different lipid and fatty acid classes are summarized below.

1.1. CLASSIFICATION OF LIPIDS AND FATTY ACIDS

Lipids are esters of fatty acids with different alcohols and insoluble in water but soluble in non polar organic solvents such as chloroform and benzene. According to Christie (2003) lipids are broadly classified into two major classes – Simple lipid and compound lipid.

A. SIMPLE LIPID

Simple Lipids are esters of fatty acids with different alcohols and carry no other substance or in other words they can give one or more different types of hydrolysis products per mole. Simple lipids or neutral lipids are non polar uncharged lipids such as hydrocarbons, wax esters, steryl esters, acylglycerols, fatty acids, sterols, prostaglandins, etc.

a. HYDROCARBONS

These are simplest type of lipids. They occur as normal branched and unsaturated chains of various lengths. The general formula of normal saturated hydrocarbon is \( \text{CH}_3(\text{CH}_2)n\text{CH}_3 \). Normally the value of ‘n’ ranges within 6-36 and sometimes may be greater.

b. WAX ESTER

They are the esters of long chain high molecular weight monohydrlic alcohol and the fatty acids are generally saturated or monounsaturated up to 30 carbons.

c. STERYL ESTERS

These are the fatty acid esters of sterols and mostly present in plasma lipoproteins and membranes. Cholesteryl esters are the commonest among steryl esters.

d. ACYLGLYCEROL

These are fatty acid esters of glycerol. They occur in three main types, \( i.e., \) monoacylglycerol, diacylglycerol and triacylglycerol. Monoacyl and diacylglycerols are respectively monoesters and diesters of glycerol and both of them have two isomeric forms. Triacylglycerols are the fatty acid triesters of glycerol. It is the most abundant simple lipid class. The fatty acids may be same or different.
e. **STEROLS**

These are the class of steroids containing a tetracyclic (cyclopentanoperhydro-phenanthrene) ring system with three rings resembling phenanthrene to which a cyclopentane ring is attached.

f. **PROSTAGLANDINS**

These are the hydroxyl or ketohydroxy derivatives of the parent C20 cyclopentane acid (prostanoic acid) and have properties like that of hormones. They are important examples of local hormones.

g. **FATTY ACIDS**

Most of the fatty acids occur as neutral fats. It usually contains an even number of carbon atoms because they are biosynthesized from 2-carbon units and occur in many diverse forms, with variation in degree, kind of branching, number of double bonds, presence of other functional groups and chain length. They are largely found in esterified forms e.g., wax esters, acylglycerols, steryl esters, phosphatides and glycolipids. Normal and saturated acids have the general formula CH₃(CH₂)nCOOH, where the value ‘n’ ranges within 4-30.

B. **COMPOUND LIPIDS**

Compound lipids are esters of fatty acids with different alcohols which in addition carry other substance like phosphate, nitrogen base, carbohydrates, and protein. According to Christie (2003) compound lipids are broadly classified into glycerophospholipids, sphingolipids and glyceroglycolipids.

a. **GLYCEROPHOSPHOLIPIDS**

These are amphipathic esters of fatty acids with a phosphate and often either a second glycerol or nitrogen base or amino acid. Phospholipids molecule has a polar head group consisting of PO₄ and two non polar hydrocarbon tails. Other than the Phosphate group in the polar head, the Phosphoglycerides may contain choline or ethanolamine or an amino acid or inositol or a second glycerol and are regarded as phosphatidylcholine or phosphatidylethanolamine or lipoaminoacid or lipositol or phosphatidylglycerol respectively. Because of amphipathic molecules, Phospholipids aggregate in aqueous media as surface monolayer, micelles, bilayers and liposomes.
b. SPHINGOLIPIDS

The phospholipids where the alcohol is glycerol is Phosphoglycerides and where the alcohol is nitrogenous alcohol sphingosine, they are called sphingolipids.

c. GLYCEROGLYCOLIPIDS

These are amphipathic esters of glycerol with a carbohydrate. The carbohydrate residue is either galactose or glucose in cerebrosides, sulphated galactose in sulphatides and different types of oligosaccharides in gangliosides and globosides. In glycolipids one or more monosaccharide residues are linked by a glycosyl linkage to a lipid part (Christie, 2003).

The lipids and their fatty acids are important for human nutrition upon consumption of fish and other fatty sources. Literature on the importance of various classes of lipids and fatty acids for human health and nutrition reveals the importance of consuming fish as a source.

1.2. IMPORTANCE OF VARIOUS CLASSES OF LIPIDS AND FATTY ACIDS

The importance of PUFA especially eicosapentansanoic acid (EPA) and docosahexanoic acid (DHA) are studied extensively by various workers. In addition to EPA(C20:5ω3) and DHA(C22:6ω3) all the studies that have been done so far on the composition of lipids in fishes have shown that there are a few important major fatty acids like myristic (C14:0), palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1ω7), oleic (C18:1ω9), linoleic (C18:2ω6), α-linolenic (C18:ω3) and arachidonic acid (C20:4ω6) and a few minor ones that are present in trace amounts by using the chromatographic techniques (Ackman, 1995; Ahmed, 1995; Arts et al., 2001; Bigger and El-Sherif, 2001; Ackman et al., 2002).

The presence of ω3 PUFA in fish oil have anti-inflammatory and immunomodulatory properties been observed on numerous occasions. Decreasing the dietary ω3/ω6 ratio inhibits T cell proliferation, lymphocyte-derived cytokine production, and cell-mediated immune response. Clinical studies have shown that fish oil supplementation has therapeutic effects in several autoimmune diseases, including rheumatoid arthritis, chronic glomerular disease (IgA nephropathy), multiple sclerosis, inflammatory bowel disease, and psoriasis (Belluzzi, 2001). ω3 PUFA differ in their potencies toward individual aspects of the lymphocyte response (Calder, 2001). EPA exerts a particularly strong influence, while DHA seems to selectively modulate the immune system by inhibiting autoimmune reactions and increasing the resistance to bacterial enterotoxins (Calder, 1999, 2001). It has been reported that a salmon diet, high in ω3 fatty acid, does not affect bleeding times, but changes the fatty acid composition of the

Electrophysiological studies have revealed the effects of long chain PUFA on visual evoked potentials and visual attention. Hals et al. (2000) have confirmed a deficiency of PUFA in children with acute brain damage. A deficit of ω3 PUFA in the diet is associated with reduced levels of DHA in the retina and brain tissue. Loss of DHA from the nerve cell membrane leads to dysfunction of the central nervous system in the form of irritability, susceptibility to stress, dyslexia, stereotypic behavior, aggressiveness, reduced learning capacity, impaired memory and cognitive functions, and extended reaction times (Carlson and Neuringer, 1999; Cunnane et al., 2000; Hals et al., 2000).

To combat the major problem of undernutrition, recent reviews of research in humans (Hornstra, 2001; Makrides and Gibson, 2001) confirm the benefits of ω3 PUPA during prenatal development, particularly for women during the third trimester of pregnancy and when breast-feeding. The infant formula should be enriched for preterm infants whose PUPA status at birth is lower than in-term neonates (Hornstra, 2001; Makrides and Gibson, 2001). Term formula should contain at least 0.2% DHA and 0.35% arachidonic acid (AA) in order to maintain the biochemical long chain PUFA status comparable with that of breastfed infants (Hornstra, 2001).

Reports have appeared in recent years suggesting that ω3 PUFA inhibit the growth of some tumors (Singh et al., 1998; Josyula and Schut, 1999). Another beneficial effect of ω3 fatty acid is the reduction of serum triglyceride and very low density lipoprotein levels (Bronsgeest-schoute et al., 1981). Normally ω3 fatty acids in comparison to ω6 fatty acids are low in human diet. Apart from green leaves and some seed oils, the main sources of ω3 fatty acids are fish oils. Hyperlipidaemia which is a major cause for atherosclerosis has been identified to be caused by an over consumption of fat, particularly SFA, in the diet. Atherosclerosis that leads to ischaemic heart disease (IHD), which is better known as coronary heart disease (CHD), is strongly related to plasma low-density lipoproteins (LDL) and is inversely related to high-density lipoproteins (HDL). The lipid particles like triacylglycerols (TG), cholesterols and phospholipids are transported in the predominantly aqueous environment of the blood as lipoproteins, which are lipid particles with a coat of amphiphilic compounds, the phospholipids and proteins. The protein moieties are known as apolipoproteins. They are classified into different density classes like chylomicrons, very low-
density lipoproteins (VLDL), LDL and HDL. Gurr and Harwood (1991) have given a comparative table on the composition and characteristics of the human plasma lipoproteins. It shows that as density increases, particle size decreases and so do the ratio of lipid to protein and the ratio of TG to PL and cholesterol.

Chylomicrons are the largest and least dense of the lipoproteins. They transport lipids of dietary origin and their principal components are TG. VLDLs are rich in TG and they transport endogenous lipids mostly TG synthesized in the liver or the intestine. The major site of synthesis is liver although some are also produced in the enterocytes. LDLs transport cholesterol to tissues where they may be required. In man they are the major carriers of plasma cholesterol. They are mostly derived from VLDL by a series of degradative steps that remove TG. So they have less TG and more of cholesterol and phospholipids. HDL carries cholesterol from peripheral cells to the liver by the process known as reverse cholesterol transport. They have very less TG, while PL and cholesterol are moderate. The major function is to remove free or unesterified cholesterol accumulated in the cell membranes and plasma lipoproteins, and transport it to the liver where it can be degraded and utilized for, among others, synthesis of bile acids. HDL\textsubscript{2} and HDL\textsubscript{3} are two subclasses of which according to Gurr and Harwood (1991), the former has a stronger inverse relationship with cardiovascular disease.

Lipids and fatty acids also play a significant role in membrane biochemistry and have direct effect on the membrane-mediated process in human such as osmoregulation, nutrient assimilation and transport (Ibrahim \textit{et al.}, 2004).

Observations on Eskimos in 1970s sparked great interest in ω3 PUFA research and today we know that both ω6 and ω3 PUFA have curative and preventive effects on cardiovascular diseases, asthma, hypertension, diabetes, cancers, brain aging, neurodevelopment in infants’ and fat glycemic control (Kinsella \textit{et al.}, 1990; Conner, 1997). In addition, the benefits of ω3 PUFA are associated with the synthesis of eicosanoids such as prostaglandins, thromboxanes, and leucotrienes (Christie, 2003). These ω3 fatty acids can be divided into three main categories: Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and Alpha-Linolenic acid (ALA). Results of clinical and epidemiological research suggest that EPA and DHA, found only in fish and sea foods, have extremely beneficial properties for the prevention of human coronary artery disease (Leaf and Weber, 1988) but both EPA and DHA cannot be synthesized in the human body and thus need dietary intake.
EPA and DHA are typically found in marine fish and originate from the phytoplankton and seaweed that are part of their food chain.

In humans, one of the most significant factors affecting the cellular membrane is dietary cholesterol and fats, which are delivered to cells throughout the body through lipoproteins (Goldstein and Brown, 2001). Contrary to common belief, the input of dietary cholesterol to serum total cholesterol is small (<10 mg/dl). However, high intakes of dietary cholesterol that is found only in animals increase the number of circulating LDLs (Grundy and Denke, 1990).

In 1996, the American Heart Association (AHA) agreed upon the British Government recommendation in 1994 showing that consumption of 1-2 fish meals/week reduces sudden death by 30-50% (Krauss et al., 2000). In 2002, the American Heart Association released a scientific statement, “Fish Consumption, Fish Oil, Omega-3 Fatty Acids and Cardiovascular Disease,” on the effects of omega-3 fatty acids on heart function (including antiarrhythmic effects), hemodynamics (cardiac mechanics) and arterial endothelial function.

The assessment of the physiological importance of different classes of lipids and fatty acids is obtained by continuous detail studies in this field which are briefly mentioned.

Many studies on fish lipid composition have been done by improved analytical methods. The role of cholesterol and lipids in atherosclerosis has been studied since the work of Anitschkow and Chalatow in 1913 (Maxfield and Tabas, 2005). Since the time the essentiality of EFAs was established in 1929, studies on nutritional and health effects of ω6 PUFA received wide attention. Studies on the effect of ω3 fatty acids rather attracted relatively little notice.

The reduced platelet aggregation and prolonged bleeding times of the Greenland Eskimos suggested an important mechanism by which ω3 PUFA could affect CHD (Dyerberg et al., 1975, 1978). Early studies of the Inuits highlighted their lower coronary mortality compared to their Danish counterparts. Their diet included a strikingly higher intake of ω3 PUFA from their marine food sources like seals and whales. The consumption of marine fish and other sea food is beneficial in reducing the risk of cardiovascular disorders was further demonstrated later (Glomset, 1985; Dyerberg, 1986; Leaf and Weber, 1988). These studies suggest that consumption of marine food stuffs resulted in lower blood cholesterol, lower TG, lower LDL- and VLDL-cholesterol, increased HDL-cholesterol, increased bleeding times, and lower rates of CHD (Kromhout et al., 1985; Shekelle et al., 1985; Dolecek and Grandits, 1991;
Kromhout et al., 1995). Kromhout et al. (1985) had shown that an intake beyond 30g of fish per day had no obvious linear benefits. On the other hand, Julius Fast (1987) mentioned in his book about the ω3 breakthrough as well talked about the good fats competing with the bad fats and the importance to minimize the intake of trans- fats and cholesterol (animal fat) while consuming enough good fats.

Putative mechanisms of dietary ω3 PUFA on lipoprotein metabolism in humans have been listed by Connor (1994) as they i) inhibit VLDL triacylglycerol synthesis, ii) decrease apoprotein-b synthesis, iii) enhance VLDL turnover with an increased fractional catabolic rate of VLDL, iv) depress LDL synthesis, and v) reduce postprandial lipidaemia. Review of studies on prevention of thrombosis shows the importance of ω3 PUFA, which affects cellular responses in platelets, monocytes and endothelial cells (Nordoy, 1994). The Zutphen study of Keli et al. (1994) was based on fish consumption and the reduced risk of stroke but Mori et al. (1994) had noted that consumption of fish lowers cholesterol only if the intake of other fats is reduced.

Silva et al. (1996) stated that fish consumption is an important modulator of fish oil efficacy and concluded that the TG lowering effect of fish oil is affected by fish consumption. Stone (1996) mentioned that reducing the intake of saturated fat and dietary cholesterol decreases the risk of atherosclerotic vascular diseases. He also notes that “although fish oil is not recommended in the treatment of hypercholesterolemia, it does have a role in the treatment of lipoprotein disorders characterized by severe hyperglyceridemia”. Epidemiological studies as well as metabolic ward studies have shown that fish and fish oils that are rich sources of ω3 fatty acids have favourable effects on CHD and improve lipid profiles in hyperlipidaemic patients. The Lugalawa study in Tanzania carried out by Pauletto et al. (1996a, b) showed similar results with fish in the diet.

Yamada et al. (2000) did a parallel study in Japan with marine fish consumption. They declared that diets rich in fish are associated with a decrease in the incidence of atherosclerosis and are related to the ω3 fatty acid content of the fish.

Long chain ω3 PUFA possesses antiarrhythmic and antithrombotic activity, reduces blood pressure and risk of brain hemorrhage, and has been used with good results in patients after angioplasty (Kromhout et al., 1985; Christensen et al., 1999; Richter, 2001; Rubba and Iannuzzi, 2001; Schacky, 2001). These effects have been seen well in women (Iso et al., 2001). Bays et al (2003) state that marine fish oils rich in ω3 fatty acids lower TG and may be
effective in combination with statins treat combined hyperlipidaemia. They have also listed a number of cardiovascular effects that are complimentary to statins.

Saha and Ghosh (1938-39, 1939-40, and 1941) did the initial investigations of the nutritional value of fishes of Bengal. Further studies on this aspect were done Ramwell et al. (1968), Sen et al. (1976a, b, 1997), Mitra et al. (1977), Nair and Gopakurnar (1977, 1978), Chetty et al. (1989), Olsen et al. (1990), Gutierrez and Da Silva (1993), Lilabati and Vishwanath (1996), Ghosh and Dua (1997), Mathew et al. (1999), Lakshmanan et al. (1999), Pal et al. (1999), Osman et al. (2001), Ackman et al. (2002), Bhuiyan et al. (2006), Visentainer et al. (2007), Velansky and Kostetsky (2008) and Majumder et al. (2013, 2015). Apart from these there is a host of other workers who have done a whole lot of work on fish lipid as a whole and a few of them are Watanabe (1982), Misra et al. (1983), Bhuiyan et al. (1986), Harris (1989), March (1993), Gopakumar (1993), Ayala et al. (1993), Ackman (1994a, b, 1995, 2000), Henderson (1996), Buzzi et al. (1997a, b), Banerjee et al. (1997), Zenebe et al. (1998), Lawan et al. (2000), Bigger and El-Sherif (2001), and Upadhya et al. (2002). A great deal of variation is reported in percentage of lipid composition in Indian major and minor carps as well as in other tropical fishes from these works. Richter (2001) has been mentioned the detail updates in this field on research on human presented at 24th World Congress of the International Society for Fat Research (ISF).

Though the overall interest in this field is rising day by day but there are so many marine fishes consumed daily which have no ready references about their nutritional quality particularly from the aspect of lipid. All of these fishes under study are very much available in the local markets though analysis of lipid and fatty acid from the body flesh of them has not been done so far. Little work on fatty acid from the fish oil samples of Anchovy (Setipinna taty) body muscle has been done by Bhuiyan et al. (2006) which has not revealed sufficient light on fatty acid composition of different lipid classes. This scarcity of data does not lead to a beneficial conclusion on behalf of the consumption of this fish. Considering the lacunae, the present study is mainly aimed at:

1. To evaluate the total lipid and relative proportions of various lipid classes from the body flesh of the fish species namely gangetic hairfin anchovy (Setipinna phasa, Hamilton 1822), khoira (Gudusia chapra, Hamilton 1822), topsia (Polynemus paradiseus, Linnaeus 1758) and pangasius (Pangasius pangasius, Hamilton 1822) which are caught in huge quantity daily for their usefulness.
2. To estimate the neutral lipid and phospholipid fractions.

3. To evaluate the distribution of different saturated, monounsaturated and polyunsaturated fatty acids among the total lipid, neutral lipid, glycolipid and phospholipid classes.

4. To calculate the ratio of \( \omega3 \) and \( \omega6 \) as well as comparison of EPA and DHA in each of the fish.

5. To derive the atherogenic index (AI) and thrombogenic index (TI) for the assessment of fat quality. These two indices were calculated according to Ulbricht and Southgate (1991).

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\text{Atherogenic index (A.I.)} = \frac{\text{Laurie acid} + 4\text{Myristic acid} + \text{Palmitic acid}}{\sum \text{PUFA} (\omega3) + \sum \text{PUFA} (\omega6) + \sum \text{MUFA}}
\]

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\text{Thrombogenic index (T. I.)} = \frac{\text{Myristic acid} + \text{Palmitic acid} + \text{Stearic acid}}{0.5 \sum \text{MUFA} + 0.5 \sum \text{PUFA} (\omega6) + 3 \sum \text{PUFA} (\omega3) + \omega3/\omega6}
\]

From these data this study aims to evaluate the serving frequency per week for all the fishes. The hypothesis of the present study is that as marine fishes follow a particular pattern of lipid and fatty acid distribution in their flesh, the fishes under study will also show the similar pattern of muscle lipid and fatty acid distribution and contain sufficient amount of \( \omega3 \) and \( \omega6 \) fatty acids.