4. **Discussion**
Lipids are the principal stored forms of energy in many organisms including fish and human. Phospholipids and sterols are major structural elements of biological membranes. Other lipids, although present in relatively small quantities, play crucial roles as enzyme cofactors, electron carriers, light absorbing pigments, hydrophobic anchors for proteins, “chaperones” to fold membrane proteins and emulsifying agents in the digestive tract, hormones, and intracellular messengers.

Lipids are the fatty acid esters of alcohol and are the primary energy depots of animals. These are used for long-term energy requirements during periods of extensive exercise or during periods of inadequate food and energy intake. Fish have the unique capability of metabolizing these compounds readily and, as a result, can exist for long periods of time under conditions of food deprivation. A typical example is the many weeks of migration by salmon in their return upstream to spawn; stored lipid deposits are burned for fuel to enable body processes to continue during the strenuous journey. In addition to locomotion, spawning, migration, etc., lipids and their constituent fatty acids are also used as an energy source for reproduction and structural components of membrane which maintains the lipid homeostasis in the fish.

Lipid homeostasis can be described as the balance between lipid uptake, transport, storage, biosynthesis, metabolism, and catabolism, and each of these processes has to be controlled independently in a specific manner, in addition to be regulated on a tissue and whole body level which intern is essential for human nutrition upon consumption of fish. The total fatty acid pool of our body is made by the total ingested fatty acids from food and as well as the fatty acids that can be synthesized. The fatty acids that are stored in our body as fat or as cell membrane phospholipids are the subject of a constant turnover. Fat molecules and phospholipids are continuously broken down, and fatty acids are continuously assembled to form fat molecules and phospholipids to maintain the lipid and fatty acid homeostasis in man.

Most of the people in the developing countries are dependent on fish as a source of animal protein. It has been estimated that about 80% of the animal protein in the diet of the people of Bangladesh are contributed by fish (Hawk and Oser, 1965). Furthermore, the fatty acids of fish lipids are rich in ω3 long chain, highly unsaturated fatty acids that have particular importance in fish and human nutrition, and are reflecting their roles in critical
physiological processes (Sikorski et al., 1990). Fish being the most important food source of these vital nutrients for man, the longstanding interest in fish lipids stems from their abundance and their uniqueness.

The results of the lipid and fatty acid classes of four marine fishes, are discussed with other freshwater and marine fishes considering biochemistry, metabolism and functions on fish physiology in relation to human nutrition.

4.1. THE TOTAL LIPID (TL)

Lipids are major sources of energy for the body, and they also provide the hydrophobic barrier particularly in fish that permits partitioning the aqueous contents of cells and sub-cellular structures. Lipids serve additional functions in the body, for example, some fat-soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis.

4.1.1. COMPARATIVE ANALYSIS ON TL OF THE FISHES OF PRESENT STUDY

The amount of TL obtained from various fish was investigated and it was observed that the value ranges between 0.6-30% (Atchison, 1975; Farkas and Csengeri, 1976; Farkas et al., 1978; Dave et al., 1976). Lambertsen (1978) classified fish according to TL content into the following 4 classes- 1. Lean (<2%): Cod, haddock. 2. Low fat (2-4%): sole, halibut, flounder. 3. Medium fat (4-8%): wild salmon. 4. High fat (>8%): herring, mackerel, sablefish, farmed salmon. The TL content in the muscle of four major fresh water food fish of India (Labeo rohita, L. calbasu, Catla catla, and Cirrhinus mrigala) was about to 1% and in Labeo bata it is 2.5% (Ackman et al., 2002). Variation in TL content was observed in different fresh water fish of commercial importance from tropical lakes in the Ethiopian Rift Vally (Zenebe et al., 1998), showing that TL content ranges between ≤ 5% to ≥ 10%. The extent of variation was more pronounced in the herbivorous (Oreochromis niloticus) than the omnivorous (Barbus sp.) and carnivorous (Clarias gariepinus) fish.

Hilsa (Tenualosa ilisha, Hamilton) is one of the important commercial fish species in Hooghly estuarine system of West Bengal. Depending on different size groups and pre-monsoon and monsoon seasons, values of TL content varies from 1.32% to 20.85%. Purely marine fish species generally have higher level of lipids; being a migratory fish, hilsa lipid content varies widely (Nath and Banerjee, 2012). The TL content was determined in edible meat of fifteen marine fish species caught on the Southeast Brazilian coast and two from East
Antarctic show that most of the fish had lipid amounts lower than 10% of their total weight (Visentainer et al., 2007). In pomfret (Pampus argenteus) TL content in the muscle is 1.43% (Chakraborty et al., 2005) which is comparatively low though it is a marine species. Similarly, in Bombay duck (Harpadon nehereus) TL content in the muscle is 2.1% (De et al., 1999).

In the present study, it was found that S. phasa and G. chapra have TL content of 12.51% and 10.72% respectively, reflecting that both of them have high fat contents than that of P. paradiseus and P. pangasius having 3.03% and 1.37% respectively (Table 1 and figure 10). P. paradiseus inhabits shallow sandy inshore areas and evidently ascends the estuary during breeding from March to June when they were collected. Since most of the times they live over sandy bottom and feed upon crustaceans (especially shrimps), small fishes and benthic organism their staple diet is not composed of very fatty marine prey species and phytoplanktons. On the other hand, the matured P. pangasius commence migration from the brackish-water and reach into the freshwater for spawning. The larvae and fry are drifted down along the water current towards the estuary where the fish resides at least for three years for maturation and migrates again for spawning (David, 1963). They feed on snails and other mollusks even gorging of mollusks, carcasses and vegetable matters. Therefore feed composition of this fish does not comprise fatty marine organisms and phytoplankton. On the contrary, being comparatively stenohaline, S. phasa and G. chapra obtain lipid rich marine food sources which results in high fat content in both of them (Majumder et al., 2015).

4.1.2. THE SOURCE, EXPORT AND IMPORTANCE OF TL IN FISH PHYSIOLOGY

4.1.2.1. THE SOURCE AND EXPORT OF TL IN FISH

Purely marine fish species generally have higher level of lipids. Many high-latitude zooplanktons can routinely contain two-thirds or more of their dry body weight as oil, largely wax esters. Capelin (Mallotus villosus) can routinely contain 20% or more of their wet body weight as oil, largely triacylglycerols. Therefore, it is self evident, that fish consuming such oil-rich prey, for example, capelin consuming zooplankton, or cod or salmon consuming capelin, are capable of efficiently digesting and assimilating large quantities of lipid, and often of depositing large quantities of oil in their body tissues (Tocher, 2003).

Lipids are exported from the intestine in the form of very-low-density lipoprotein (VLDL)-like particles in the lumen, as has been directly observed in freshwater species. The
lipid load and degree of unsaturation affects lipoprotein production. In fish the majority of the intestinal lipoproteins are transported via the lymphatic system before appearing in the circulatory system and being delivered to the liver (Sheridan et al., 1985).

Teleosts have a major plasma protein with the approximate size, solubility, and electrophoretic mobility of mammalian serum albumin (De Smet, 1978). This protein presumably functions to transport free fatty acids in the blood from adipose tissue depots to peripheral tissues under appropriate physiological conditions, as it does in mammals (Sheridan, 1988). Fish plasma contains a similar range of lipoproteins to mammalian plasma, namely, chylomicrons, VLDL, low-density lipoproteins (LDL), and high-density lipoproteins (HDL) (Sheridan, 1988; Babin and Vernier, 1989). The lipid composition of lipoproteins varies among different fish species. The major neutral lipids triacylglycerol and steryl esters, decreases from chylomicrons through VLDL to LDL and HDL, whereas the proportion of surface components such as phospholipids, free cholesterol, and protein increases (Babin and Vernier, 1989). Thus, triacylglycerols constitute about 85%, 52%, 22%, and 11% of chylomicrons, VLDL, LDL and HDL, respectively, in trout, whereas phospholipids account for 8%, 19%, 27%, and 32% of the total weight of those lipoproteins respectively (Babin and Vernier 1989). Generally, similar compositions were found in serum VLDL, LDL, and HDL from Pacific sardine (Sardinops caerulea), HDL from pink salmon and chum salmon (Oncorhynchus keta), and serum lipoproteins from red sea bream and sea bass (Dicentrarchus labrax) (Ando and Hatano, 1988; Iijima et al., 1995; Santulli et al., 1997).

Another lipoprotein class in fish plasma is vitellogenin, which is only found in mature oviparous females or estrogen-injected fish (Wallace, 1985). Therefore, the assay of plasma vitellogenin level is useful in determining sexual maturity in female fish through blood sampling alone (Susca et al., 2001). Vitellogenin has a density higher than HDL generally containing about 80% protein and 20% lipid, and has also been termed very high-density lipoprotein I (VHDL I). The cDNA of the vitellogenin protein has been cloned from several species of fish, including fathead minnow (Pimephales promelas) (Korte et al., 2000), tilapia (Oreochromis aureus) (Lim et al., 2001), and haddock (Melanogrammus aeglefinus) (Reith et al., 2001) which were further utilized to understand the synthesis, transport and role of this lipoprotein in fish. Vitellogenin is synthesized in the liver and is transported to the ovary during the first stage of oogenesis termed vitellogenesis (Wallace, 1985). Vitellogenin is taken up by receptor-mediated micropinocytosis into the developing
oocytes where it is cleaved into phosvitin and lipovitellin, phosphate- and lipid-rich proteins, respectively (Selman and Wallace, 1982).

4.1.2.2. THE IMPORTANCE OF LIPID IN FISH PHYSIOLOGY

Lipids and their constituent fatty acids are, along with proteins, the major organic constituents of fish. Muscle lipids in fish are used as an energy source for locomotion, stored and later transported to gonads for reproduction, and utilized during spawning migration and actual spawning. These processes proceed differently in various species or even in subspecies (e.g., Baltic herring and sprat) and are quantitatively and qualitatively different in males and females of the same species, explaining the different lipid contents and compositions between sexes (Kolakowska, 1991a) which maintains the lipid homeostasis in the fish.

4.2. THE TL CLASSES

In the present study the TL obtained from the fish muscle is separated into three major fractions namely- neutral lipid, phospholipid and glycolipid. The neutral lipids are further fractionated into triacylglycerol (TG), 1-O- alkyl-diacyl glycerol (ADAG), sterol (ST), hydrocarbon (HC), wax ester (WE) and steryl ester (SE). Again, the phospholipids are classified into cardiolipin (CL), phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SPH) and phosphatidylinositol (PI).

4.2.1. NEUTRAL LIPID (NL)

Mono-, di-, and triacylglycerols consist of one, two, or three molecules of fatty acid esterified to a molecule of glycerol. Fatty acids are esterified through their carboxyl groups, resulting in a loss of negative charge and formation of "neutral lipid."

4.2.1.1. COMPARATIVE ANALYSIS ON DIFFERENT NL FRACTIONS OF THE FISHES UNDER STUDY

Among the TL fractions, the NL contents of all the fishes under study are predominant. The comparative distributions of different NL fractions are represented in table 2 and figure 11. The six components of NL of all fish are showing the same pattern of distribution where Triacylglycerols (TG) content is found to be abundant. *P. pangasius* has maximum amount of TG (58.42%) and *P. paradiseus* has minimum (52.53%). In *S. phassa* it is 57.92% and in *G. chapra* little less (53.18%). TG is the storage lipids in almost all commercial fish species (Kolakowska and Kolakowski, 1990). After attaining maturity, *P. pangasius* commence migration from the brackish-water and reach into the freshwater for spawning (David, 1963) and that is the purpose of containing high TG in their muscles.
Similarly, as determined over two annual cycles, the Baltic herring muscle consisted of 51 to 88% TG with increasing lipid content in muscles (Kolakowska and Kolakowski, 1990; Kolakowska et al., 2000, 2001). Among the other fractions of NL, in all the fish species significant amount of ADAG is found.

Wax esters may also be a major component of some species' flesh and/or livers (Nichols et al., 2001a). In all the fishes under study wax esters are found in little amounts but in some Antarctic aquatic animals, including *Euphausia crystalorophias* and *Euphausia triacanta*, wax esters are the storage lipids (Kolakowska, 1987, 199lb). Wax esters are deemed more stable and are more suitable for long-term storage of energy compared to short-term energy stores such as TG. In addition, wax esters aid in buoyancy and occur in higher amounts in roe lipids (Sen et al., 1997). A number of nutraceutical and cleaning products have been produced from wax esters, glycerol esters, and squalene extracted from fish (Nichols et al., 2001b).

Fish sterol content is relatively stable and ranges between 40 and 60 mg per 100 g edible fish muscle (Ackman, 1994b; Krzynowek et al., 1990); 250 to 650 mg per 100 g roe (Sikorski et al., 1990); and 480 to 1150 mg per 100 g oil in livers of cod, herring, menhaden and salmon (Kinsella, 1987; Kennish et al., 1992). In *S. phasial* ST and steryl esters contents are 2.74% and 0.15% and in *G. chapra* they are 3.87% and 0.18% respectively. In a study of Australian seafood, the highest sterol contents (120 to 160 mg/100 g) were recorded in shellfish such as prawns, squid, octopus, and scallops (Nichols et al., 1998). In fin fish 95% of the ST is cholesterol (Sikorski and Kolakowska, 2003). Cholesterol is a major component of plasma membrane and metabolic precursor of steroid hormones and bile acids. Cholesterol is the precursor of all classes of steroid hormones: glucocorticoids (for example, Cortisol), mineralocorticoids (for example, aldosterone), and sex hormones- androgens, estrogens and progesterone.

A consumer survey on 10 species of most-preferred marine fish for daily consumption in Malaysia identified that TL content is less than 6% and total cholesterol content was 37.1-49.1 mg/100g (Osman et al., 2001). Cholesterol in Indian fish and shellfish has recently been tabulated by Mathew et al. (1999). This may be of further interest in India where there is a growing incidence of cardiovascular disease (Mohan et al., 2001; Yusuf and Ounpuu, 2001).
4.2.1.2. THE NL FRACTIONS AND THEIR IMPORTANCE IN FISH PHYSIOLOGY

4.2.1.2.1 SYNTHESIS, STORAGE AND MOBILIZATION OF TRIACYLGLYCEROLS (TG)

Triacylglycerols consist of three molecules of fatty acids esterified to the three alcohol groups of glycerol. When esterified, these positions are termed sn1, sn2 (middle position), and sn3 due to the asymmetry induced by the enzymatic esterification. In fish lipids, generally saturated and monounsaturated fatty acids are preferentially located in the sn1 and sn3 positions, whereas PUFA are preferentially located in the sn2 position. However, many exceptions exist to this general rule; for example, tridocosahexaenoyl (tri 22:6 ω3) glycerol can be a major component of the triacylglycerols in the eye lipids of some fish (Nicol et al., 1972).

4.2.1.2.1.1 TRIACYLGLYCEROL SYNTHESIS

In mammals, triacylglycerols are formed by the sequential esterification of two fatty acids to glycerol-3-phosphate to form lyso-phosphatidyl A and phosphatidyl A catalyzed by glycerophosphate acyltransferase, followed by cleavage of the phosphate group (via phosphatidate phosphatase) to form diacylglycerol and the esterification of a further fatty acid (via diacylglycerol acyltransferase) to form triacylglycerol. The pathway of de novo triacylglycerol biosynthesis has not been studied extensively in fish, but the little evidence available suggests that the pathways are generally the same in fish as in mammals (Sargent, 1989).

4.2.1.2.1.2. STORAGE OF TRIACYLGLYCEROL

During feeding, excess dietary fatty acids are exported from the liver in the form of lipoproteins (VLDL) and are accumulated and stored in the form of triacylglycerols in specific lipid storage sites. The primary site for long-term storage in many fish is the mesenteric adipose tissue, although some fish also store significant amounts of fat within the white (light) muscle (adipose tissue within the myosepta) and between skin and muscle, which can account for a large proportion of the fish’s total reserves (Henderson and Tocher, 1987). Red (dark) muscle, which usually has higher lipid content than white muscle, contains most of the lipid as finely dispersed oil droplets within the muscle fibers themselves (Sheridan, 1994).

4.2.1.2.1.3. MOBILIZATION OF TRIACYLGLYCEROL

Triacylglycerols are stored in adipose tissue fundamentally as a long-term source of energy that can be used when the energy requirements of the animal exceed the energy available from the diet, particularly when the energy requirements of the animal are very
Apart from starvation, a particular example of this in fish is during reproduction when the production of very large numbers of gametes, particularly eggs, during the relatively short period of reproduction, is very energy intensive. A further example is the migration, often long distance that precedes reproduction in many fish because the energy requirements of the swimming red muscles are provided largely by fatty acids. Lipid is probably mobilized initially from the main adipose tissue, although in the longer term it will be also mobilized from the secondary lipid storage sites such as muscle and liver. A key enzyme in triacylglycerol mobilization is the “hormone-sensitive” lipase (HSL) whose activity is regulated by various hormones through reversible phosphorylations by the action of kinases and phosphatases under the influence of various activators and inhibitors. Direct enzymatic studies on HSL in fish are few, but the process of triacylglycerol mobilization is known to be under hormonal control, at least in the liver, indirectly implying the presence of a HSL in fish (Sheridan, 1994). In starvation or periods of non feeding, lipid mobilization from liver and adipose tissue in fish, as in mammals, is under β-adrenergic control, with adrenalin (epinephrin) and noradrenalin (norepinephrine) stimulating triacylglycerol hydrolysis and an increase in plasma free fatty acids in salmonids and various other fish species, although the potency of the two catecholamines varies between different species (Sheridan, 1994; Fabbri et al., 1998).

TG is the storage lipids in almost all commercial fish species. The TG content increases with increasing lipid content in muscles. TG is a major lipid class in the diet of fish. In mammalian gut TG hydrolysis is affected by either pancreatic lipase-colipase system (EC 3.1.1.3) or bile salt activated lipase (EC 3.1.1.1). Evidence points to the presence of both of the enzyme systems in many teleost fish (Olsen and Ringoe, 1977; Leger et al., 1977, 1979). The presence of both of the enzyme systems simultaneously in trout by Leger (1985) with Tocher and Sargent (1984) suggests that one enzyme predominantly hydrolyzed TG and other one hydrolyzed wax and steryl esters.

4.2.1.2.2. ADAG
Mono- and di- acylglycerols are other lipid components of the various membranes of cellular organelles, and indeed they are the most abundant lipids in all photosynthetic tissues, including those of higher plants, marine algae and certain bacteria. C16 or C18 fatty acids tend to be found mostly in ADAG.
4.2.1.2.3. STEROLS

The most important simple lipid (*i.e.*, a lipid not containing fatty acids) in all animals, including fish, is cholesterol. This is the most common of the tetracyclic hydrocarbon compounds, collectively called sterols, and can exist unesterified as an essential component of cell membranes or in a neutral lipid storage form esterified to a fatty acid. They are unsaponifiable, alicyclic hydrocarbons, having a fused, tetracyclic, cyclopentanoperhydrophenanthrene ring system in their molecules which may be esterified with fatty acids to form steryl esters. Cholesterol is a major component of plasma membrane and metabolic precursor of steroid hormones and bile acids.

4.2.1.2.4. WAX ESTERS

Wax esters constitute another class of neutral lipid consisting of a single molecule of fatty acid esterified to a single molecule of a fatty alcohol. This lipid class is very abundant in marine zooplankton, particularly in calanoid copepods and in euphausiids (red feed and krill, respectively) that form major natural foods for many species of marine fish. Wax esters can also be present in considerable amounts in the body tissues and eggs of some fish species. The fatty acids of marine wax esters can be of a variety of chain lengths and can be saturated, monounsaturated, or polyunsaturated. However, the fatty alcohols are generally saturated or monounsaturated and, in the case of high-latitude marine zooplankton, the alcohol moieties can be very rich in 20:1ω9 and 22:1ω11 structures. Wax esters are converted to triacylglycerols during the process of digestion and absorption in the intestinal tissue of zooplanktonivorous fish. Thus, the large amounts of 20:1ω9 and 22:1ω11 fatty acids in the triacylglycerols of many fish oils from the northern hemisphere, that is, sand eel, herring, and capelin oils, are derived directly from the oxidation of the corresponding fatty alcohols ingested from zooplankton wax esters (Sargent and Henderson, 1995). These fatty acids are present in much lower percentages in fish oils from the southern hemisphere, most notably anchovy oils, which are correspondingly richer in ω3 PUFA, especially 20:5ω3 (Sargent and Henderson, 1995).

Wax esters are deemed more stable and are more suitable for long-term storage of energy compared to short-term energy stores such as TG. In addition, wax esters aid in buoyancy and occur in higher amounts in roe lipids (Sen *et al.*, 1997). A number of nutraceutical and cleaning products have been produced from wax esters, glycerol esters, and squalene extracted from fish (Nichols *et al.*, 2001b). Many zooplanktons can routinely
contain two-third or more of their body weight as oil, large wax esters. Capelin (*Mallotus villosus*) can routinely contain 20% or more of their wet body weight as oil, largely triacylglycerol. Therefore it is evident that fish consuming such oil-rich prey, for example, capelin consuming zooplankton, or cod or salmon consuming capelin, is capable of efficiently digesting and assimilating large quantities of lipid and depositing that in their body tissue (Tocher, 2003).

The fatty acid of marine wax esters can be a variety of fatty acids and can be saturated, monounsaturated or polyunsaturated. Wax esters are converted to triacylglycerols during the process of digestion and absorption in the intestinal tissue of zooplanktonivorous fish. Thus, the large amounts of 20:1ω9 and 22:1ω11 fatty acids in the triacylglycerols of many fish oils are derived directly from the oxidation of the corresponding fatty alcohols ingested from zooplankton wax esters (Sergent and Henderson, 1995). It is not clear whether fish possesses a specific wax ester hydrolase because no specific enzyme has been purified or characterized (Tocher, 2003). Wax ester hydrolase activity could not be separated from TG hydrolase activity in carp hepatopancreas (Kayama et al., 1979).

4.2.1.2.5. STERYL ESTERS

The unsaponifiable, alicyclic sterols, having a fused, tetracyclic, cyclopentanoperhydro-phenanthrene ring system in their molecules, may be esterified with fatty acids to form steryl esters.

4.2.1.3. IMPORTANCE OF NL ON HUMAN PHYSIOLOGY

The ring structure of cholesterol cannot be metabolized to CO₂ and H₂O in humans. Rather, the intact sterol nucleus is eliminated from the body by conversion to bile acids and bile salts, which are excreted in the feces, and by secretion of cholesterol into the bile, which transports it to the intestine for elimination. Some of the cholesterol in the intestine are modified by bacteria before excretion. The primary compounds made are the isomers coprostanol and cholestanol, which are reduced derivatives of cholesterol. Together with cholesterol, these compounds make up the bulk of fecal sterols.

Cholesterol is transported from the liver to extrahepatic tissues mainly in low density lipoprotein particles (LDL) of plasma and from extrahepatic tissues to the liver in high-density lipoprotein particles (HDL). In human, the average level of cholesterol is 175 mg/100 ml of blood plasma. A high plasma LDL-cholesterol may lead to atherosclerosis by depositing cholesterol plaques on extrahepatic vascular walls.
Atherosclerosis is a progressive disease that begins as intracellular lipid deposits in the smooth muscle cells of the inner arterial wall. These lesions eventually become fibrous, calcified plaques that narrow and even block the arteries. The resultant roughening of the arterial wall promotes the formation of the blood clots, which may also occlude the artery. A blood flow stoppage, known as infarction, causes the death of deprived tissue. Although atheromas can occur in different arteries, they are most common in coronary arteries resulting ultimately myocardial infarctions (MI) or heart attacks. In normal individuals the LDL-Cholesterol are taken up by the cell surface LDL receptors via receptor-mediated endocytosis. The presence of excess cholesterol inhibits the synthesis of both cholesterol and LDL receptor within the cell. Cholesterol is converted to cholesteryl ester that is excreted mainly in the bile as in the non-aqueous central core of micelles formed by bile salts and PC.

Individuals with familial hypercholesterolemia have high level of LDL-Cholesterol in their plasma due to lack of LDL receptors. The excess LDL becomes oxidized and is taken up by macrophages by their scavenger receptors. Within the cells oxygen radicals convert the LDL’s unsaturated fatty acids to aldehydes and oxides that react with its lysine residues. Thus accumulation of excess cholesterol converts macrophage into foam cell. The situation results in the deposition of cholesterol in their skin and tendons as yellow nodules known as xanthomas ultimately leading to MI (Voet and Voet, 1995). Therefore, fish with high cholesterol content should not be preferred as healthy.

4.2.2. PHOSPHOLIPIDS (PL)

Phosphoglycerides are a major class of polar lipid characterized by a common backbone of phosphatidic acid, which is L-glycerol 3-phosphate containing two esterified fatty acids. Saturated and monounsaturated fatty acids are preferentially esterified on position sn-1 of the L-glycerol 3-phosphate with PUFA preferentially esterified on position sn-2. Phosphatidic acid is esterified to the “bases” choline, ethanolamine, serine, and inositol to form the major phosphoglycerides of animal, including fish tissues, viz. phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol.

4.2.2.1. COMPARATIVE ANALYSIS ON DIFFERENT PL FRACTIONS OF THE FISHES OF PRESENT STUDY

A typical fish polar lipid fraction contains about 60% phosphatidylcholine (PC), 20% phosphatidylethanolamine (PE), and several percent phosphatidylserine and sphingomyelin, while the remainder is composed of other minor phospholipids. In fish the phospholipid PC:
PE ratio is generally 2-3:1 (Sikorski and Kolakowska, 2003). Calculating the PC: PE ratio from table 2 it is found that in *S. phasa* and *P. paradiseus* this PC: PE ratio is 3.62:1 and 3.35:1 respectively, slightly higher than the normal range. In *G. chapra* and *P. pangasius* it is 2.58:1 and 3.0:1 respectively, belonging within normal range. The PC: PE ratio of bream, a freshwater fish was as low as 1.3 to 1.9:1 (Kolakowska et al., 1993, 2000) but in sting ray it is 4.28:1 (Pal et al., 1999). A PC: PE ratio of approximately 2:1 was recorded in phospholipids of the light and dark muscle of trout (Ingemansson et al., 1991). Various cod tissues were found to differ in their phospholipid composition. The PC: PE ratios of white and dark muscle of the cod were 3.5:1 and 2.7:1, respectively (Lie and Lambertsen, 1991). Invertebrate phospholipids contain less PC than those from vertebrates.

4.2.2.2. BIOSYNTHESIS, DIGESTION, FRACTIONS AND FUNCTION OF PHOSPHOLIPIDS IN FISH

Since phospholipids are typically the other main lipid class found in fish flesh, the leaner the fish and the higher the proportion that phospholipids contribute to total lipids. For this reason, phospholipids are the major lipid class in most Australian fish, and in mollusks and crustaceans, all of which are typically lean. In contrast to finfish, which tend to store lipid as TG, an increase in the lipid content of shellfish is usually due to an accumulation of polar lipids (Nichols et al., 1998).

4.2.2.2.1. BIOSYNTHESIS OF PHOSPHOLIPIDS IN FISH

The pathways of *de-novo* phosphoglyceride biosynthesis have not been studied extensively in fish. However, the existing evidence strongly suggests that pathways are essentially the same in fish as in higher terrestrial mammals (Sargent et al., 1989). The activity of glycerol-3-phosphate acyltransferase was demonstrated in the liver of rainbow trout (Holub et al., 1975a). The presence of cytidine diphosphate (CDP)-choline-1,2-diacylglycerol choline phosphotransferase activity has been demonstrated in the microsomes of trout liver (Holub et al., 1975b) and brain, and liver from goldfish (*Carassius auratus*) (Leslie and Buckley, 1975). However, the *de novo* pathways of phosphoglyceride biosynthesis were investigated in trout hepatocytes and the activities of CDP-choline and CDP-ethanolamine phosphotransferases, PE-methyltransferase (PE→PC) and PS-decarboxylase (PS→PE) demonstrated (Hazel, 1990). The synthesis of steryl esters has not been studied in fish, although the biosynthesis of wax esters has been demonstrated in the liver of wax ester-rich myctophid species of fish (Seo et al., 2001).
4.2.2.2. DIGESTION OF PHOSPHOLIPIDS IN FISH

The dietary phosphoglycerides are digested by pancreatic or intestinal phospholipases, resulting in the formation of 1-acyl lysoglycerophospholipids and free fatty acids that are absorbed by the intestinal mucosal cell (Henderson and Tocher, 1987; Sargent et al., 1989). Phospholipase A^2 activity in carp heteropancreas was found to be distributed in all subcellular fractions (Mankura et al., 1986). Phosphoglycerides are the most common of the phospholipids than the sphingosine containing sphingolipids.

Sphingolipids are a group of complex polar lipids that contain as their backbone the long chain amino alcohol sphingosine, or a related base. In sphingolipids, a long chain, generally saturated or monounsaturated fatty acid, for example, 24:1ω9, is linked to the amino group of sphingosine to form a ceramide, and different polar head groups are attached to sphingosine’s primary alcohol group. For example, sphingomyelin contains phosphocholine esterified to the alcohol group of sphingosine. An important group of sphingolipids are the cerebrosides in which the alcohol group of the sphingosine is linked to one or more sugars, including glucose and galactose.

4.2.2.2.3. THE PHOSPHOLIPIDS FRACTIONS IN FISH

There were altogether five components present in the PL represent in table 2 and figure 12, of which phosphatidylcholine (PC) is major in all cases. The presence of higher percentage of PL fraction was reported in the eggs of many fresh and marine fish species namely O. pabda and W. attu (Mukhopadhyay and Ghosh, 2007), Cyprinus carpio (Mukhopadhyay and Ghosh, 2003), Notopterus pallas (Mukhopadhyay et al., 2004) and Theragra chalcogramma (Bechtel et al., 2007).

4.2.2.2.3.1. CARDIOLIPIN

Two molecules of phosphatidic acid esterified through their phosphate groups to an additional molecule of glycerol are called cardiolipin (diphosphatidylglycerol). Cardiolipin is an important component of the inner mitochondrial membrane and bacterial membranes.

4.2.2.2.3.2. PHOSPHATIDYLCHOLINE (PC)

During embryonic development, the specific classes utilized depends largely on the type of eggs, with neutral lipid-rich eggs primarily utilizing neutral lipids as in red sea bream, red drum, striped bass (Chu and Ozkizilcik, 1995), and Sturgeon (Gershanovich, 1991), whereas phosphoglyceride-rich eggs such as those from herring, cod, and African catfish primarily utilized PC (Sargent et al., 1989; Verreth et al., 1994). Therefore, the catabolism of
phosphoglycerides for energy may be a common characteristic of fish eggs that are rich in phosphoglycerides. Consistent with this, PC was primarily catabolized in the phosphoglyceride-rich eggs of halibut and plaice, but not in turbot eggs where neutral lipids account for more than 50% of total lipid (Rainuzzo et al., 1992; Finn et al., 1995; Ronnestad et al., 1995). In addition PC and bile salts are quantitatively the most important organic components of bile.

4.2.2.3.3. PHOSPHATIDYLETHANOLAMINE (PE)

During embryogenesis, the conservation and/or synthesis of PE relative to PC is seen, as reported in the phosphoglyceride-rich eggs of cod (Fraser et al., 1988), plaice and halibut (Rainuzzo et al., 1992; Rainuzzo, 1993; Ronnestad et al., 1995), the neutral lipid-rich eggs of turbot (Rainuzzo et al., 1992), Senegal sole (Mourente and Vazquez, 1996), dentex (Mourente et al., 1999), and Atlantic salmon and the freshwater species, pike and African catfish (Verreth et al., 1994; Desvilettes et al., 1997). This results in a decrease and normalization of the PC: PE ratio as development proceeds, from the high values seen in most fish eggs, particularly from marine species, to values normally observed in fish tissues. This is particularly the case in the phosphoglyceride-rich eggs, dominated by PC, where PC is catabolized during embryogenesis (Henderson and Tocher, 1987).

4.2.2.3.4. SPHINGOMYELIN

The backbone of sphingomyelin is the amino alcohol sphingosine rather than glycerol. A long-chain fatty acid is attached to the amino group of sphingosine through an amide linkage, producing a ceramide, which can also serve as a precursor of glycolipids. The alcohol group at carbon 1 of sphingosine is, esterified to phosphorylcholine, producing sphingomyelin, the only significant sphingophospholipid. Sphingomyelin is an important constituent of the myelin of nerve fibers.

4.2.2.3.5. PHOSPHATIDYL INOSITOL (PI)

PI is synthesized from free inositol and CDP-diacylglycerol. PI is an unusual phospholipid in that it often contains stearic acid on carbon 1 and arachidonic acid on carbon 2 of the glycerol. PI, therefore, serves as a reservoir of arachidonic acid in membranes and, thus, provides the substrate for prostaglandin synthesis when required. PI plays a very important role in signal transmission across membranes. The phosphorylation of membrane-bound PI produces polyphosphoinositides, for example, Phosphatidylinositol 4, 5-bisphosphate or PIP₂. The degradation of PIP₂ by phospholipase C occurs in response to the
binding of a variety of neurotransmitters, hormones, and growth factors to receptors on the cell membrane. The products of this degradation, inositol 1, 4, 5-triphosphate (IP$_3$) and diacylglycerol (DAG), mediate the mobilization of intracellular calcium and the activation of protein kinase C, respectively, which act synergistically to evoke specific cellular responses. Signal transmission across the membranes thus accomplished by PI. Specific proteins can be covalently attached via a carbohydrate bridge to membrane-bound PI.

4.2.2.2.4. IMPORTANCE OF PL IN FISH

4.2.2.2.4.1. MEMBRANE STRUCTURE AND FUNCTION

Phosphoglycerides and their fatty acid compositions have a major and very well-established role in maintaining the structure and function of cellular biomembranes. Although metabolism is a very dynamic situation, the membrane phosphoglyceride and fatty acid compositions are relatively more stable than triacylglycerol (fish oil) compositions provided the environmental conditions and diet are reasonably constant (Henderson and Tocher, 1987; Sargent, 1989; Sargent et al., 1989, 2002; Tocher, 1995). PUFA in phosphoglycerides are very susceptible to attack by oxygen (oxygen radicals) and other organic radicals. The resultant oxidative damage can have serious consequences for cell membrane structure and fluidity, with potential pathological effects on cells and tissues. However, Superoxide dismutase (SOD), catalase or peroxidases such as glutathione peroxidase (Gpx) detoxifies H$_2$O$_2$ arising as a byproduct of fatty acid oxidation (Tocher, 2003).

4.2.2.2.4.2. ROLE OF PL IN EMBRYONIC DEVELOPMENT

During embryogenesis lipid utilization occurs to a greater extent after hatching, particularly in species with neutral lipid-rich eggs, possibly reflecting the greater energy demands of the mobile, free-swimming yolk sac larvae when compared with the embryonic egg phase. Two main patterns of lipid class utilization are apparent, related to egg lipid compositions. Phosphoglyceride-rich eggs tend to utilize phosphoglycerides, particularly PC, whereas neutral lipid-rich eggs utilize primarily triacylglycerols and also steryl and wax esters where present. Irrespective of that class, catabolism of lipids results in the release of free fatty acids which can either be utilized for energy or reacylated back into lipid pools for other uses which, during embryogenesis and early larval development, can be for the formation of rapidly developing larval tissues (Tocher, 2003).
4.2.3. GLYCOLIPIDS (GL)

Glycolipids (GL) are fatty acid esters of sphingosine, carrying carbohydrate in addition. In *S. phasa* only 0.72%, in *G. chapra* 2.24%, in *P. paradiseus* 4.47% and in *P. pangasius* maximum 11.98% GL were found. In Amadi (*Coilia reynaldi*), *Boleophthalmus boddaerti* and *Dasyatis bleekeri* GL contents were found in moderately higher percentage - 22.08% (Majumder *et al.*, 2013), 26.4% (Banerjee *et al.*, 1997), and 22.9% (Pal *et al.*, 1999), respectively.
4.3. THE FATTY ACIDS

Fatty acids are aliphatic monocarboxylic acids derived from, or contained in esterified form in an animal or vegetable fat, oil, or wax. Natural fatty acids commonly have a chain of 4-28 carbons (usually unbranched and even numbered), which may be saturated or unsaturated. Fatty acids exist free in the body (that is, they are unesterified) and, also are found as fatty esters in more complex molecules, such as triacylglycerols. Low levels of free fatty acids occur in all tissues, but substantial amounts sometimes can be found in the plasma, particularly during fasting. Plasma free fatty acids (transported by serum albumin) are in route from their point of origin (triacylglycerol of adipose tissue or circulating lipoproteins) to their site of consumption (most tissues). Free fatty acids can be oxidized by many tissues—particularly liver and muscle to provide energy. Fatty acids are also structural components of membrane lipids, such as phospholipids and glycolipids. Fatty acids are attached to certain intracellular proteins to enhance the ability of those proteins to associate with membranes. Fatty acids are also precursors of the hormone-like prostaglandins. Esterified fatty acids, in the form of triacylglycerols stored in adipose cells, serve as the major energy reserve of the body.

4.3.1. COMPARATIVE ANALYSIS ON MAJOR FINDINGS ON DIFFERENT FATTY ACID CLASSES FROM THE FISHES UNDER STUDY

The common saturated fatty acids (SFA) are straight-chain even-numbered acids containing 12-24 carbon atoms, although all the possible odd and even-numbered homologues with 2-30 or more carbon atoms have been found in nature. C4 to C12 acids are found mainly in milk fats.

Lauric acid (12:0) is a minor component of saturated fatty acid and not detected in S. phasa and G. chapra, but is present in little amount in P. paradiseus and much higher amount in P. pangasius.

Myristic acid (14:0) is a minor component of most animal lipids, but is present in major amounts in seed oils of the family Myristicaceae. In all the fishes of present study, myristic acid is predominant in NL but much higher amount in G. chapra.

Palmitic acid (16:0) is probably the commonest saturated fatty acid and is found in virtually all animal and plant fats and oil. In fish, Palmitic acids particularly act as a part of defense mechanism against microbial infections by secreting from epithelia of the fish skin as
anti-microbial molecules (Bergsson, 2005). In all fishes under study this acid is predominant saturated fatty acid and maximally found in GL of *S. phasa*.

Stearic acid (18:0) is also relatively common and may on occasion be more abundant than palmitic acid, especially in complex lipids. In *S. phasa* and *G. chapra* this acid is moderately present and maximally found in GL but in *P. paradiseus* and *P. pangasius* this acid is maximally found in PL.

C15 to C19 odd-chain acids can be found in only trace amounts in most animal lipids but can occur in larger quantities in certain fish flesh or in bacterial lipids (Christie, 2003). The presence of significant level of odd numbered fatty acids (15:0 and 17:0) in all of the fishes under study is interesting. Presence of significant amount of odd numbered fatty acids is also reported in *Mugil cephalus* (Iyenger and Schlenk, 1967) and roe of *Mugil parsonia* (Sen *et al.*, 1997). Decanoic and higher saturated fatty acids are solids at room temperature. Because of the lack of functional groups other than the carboxyl group, they are comparatively inert chemically (Christie, 2003).

Straight chain monounsaturated fatty acids (MUFA) of 15, 16, 17, 18, 22, 24 carbon atoms containing one double bond have been characterized from all fishes under study. In addition, 14:1 is found in *S. phasa* and *P. paradiseus* and 20:1(*ω*9) is present in *P. paradiseus* and *P. pangasius*. Monoenoic acids with double bonds in the cis- and trans-configuration are known but trans- are comparatively rare. Palmitoleic acid (16:1, *ω*7) is a component of most animal fats and present in quite high concentration in these fishes. There is a family of polyunsaturated fatty acids derived from palmitoleic acid, and odd-PUFA have been found in some fish oils.

Oleic acid or 18:1 (*ω*9) is found to be the predominant monoenoic acid in all cases. Oleic acid can also be the primary precursor of a family of PUFA. Shorter-chain monoenoic acids are common constituents of milk fats but are rarely found in significant amounts in other tissues. C20, C22 and odd-chain monoenoic acids are minor components of most animal lipids, but are found in appreciable quantities in certain fish oils (Christie, 2003). Biosynthetic relationships are, obvious as several components may arise by chain elongation or chain shortening of a common precursor as illustrated in figure 22.

In *S. phasa* only the dienoic linoleic acid or 18:2 is present in minute quantity but in *G. chapra*, 16:2 and 18:2 both are present. In *P. paradiseus* and *P. pangasius* 16:2, 18:2, and 20:2 are present. Linoleic acid or 18:2ω6 (*cis*-9, *cis*-12-octadecadienoic acids) is the
commonest and simplest fatty acid among dienes and is found in most plant and animal tissues. It is an essential fatty acid (EFA) in animal diets as it cannot be synthesized by the animal yet is required for growth, reproduction and healthy development (Holman, 1968). In animal tissues, it is the precursor of a family of other fatty acids discussed in zebra fish, tilapia, salmon, etc. (Tocher et al., 1997, 2001a, 2001b). The requirement of this desaturation and elongation depends partly on habitat. In all these fishes under study linoleic acid is present but unlike the freshwater fish, marine species cannot convert C-18 to C-20\(\omega_6\) PUFA (Sargent et al., 1999). So they have to depend on dietary sources for higher chain \(\omega_6\) PUFA. Being marine migratory \textit{P. paradiseus} and \textit{P. pangasius} show trace amount of 20:2 DUFA. In addition to 18:3\(\omega_6\) and 20:4\(\omega_6\) the other intermediates of \(\omega_6\) conversion such as, 20:3\(\omega_6\) and 22:5\(\omega_6\) fatty acids are also present in \textit{P. paradiseus} and \textit{P. pangasius}. In stenohaline \textit{S. phasa}, 22:5\(\omega_6\) fatty acid is not detected and 20:3\(\omega_6\) fatty acid is absent in both \textit{S. phasa} and \textit{G. chapra}. The source of these fatty acids may be dietary or may be a result of active elongase system from 18:2\(\omega_6\)in \textit{P. paradiseus} and \textit{P. pangasius}.

Linolenic acid or 18:3\(\omega_3\) (\textit{cis}-9, \textit{cis}-12, \textit{cis}-15-octadecatrienoic acid) is a major component of plant lipids, particularly of the photosynthetic tissues, but it is rarely a significant constituent of fish lipids. It is extremely important as the primary precursor of other polyunsaturated fatty acids. Linolenic acid and/or polyunsaturated fatty acids of the \(\omega_3\) family are essential fatty acids in fish (Sinnhuber et al., 1972). In all the fishes under present work, this acid is present.

The metabolic conversion of alpha-linolenic acid (LNA, 18:3\(\omega_3\)) to its longer-chain products via the various desaturation/elongation reactions is depicted in figure 22. This figure also shows the conversion of LA to its corresponding \(\omega_6\) PUFA products including arachidonic acid (AA, 20:4\(\omega_6\)) which, like EPA plus DHA, accumulates in various tissues and cells in the body. In contrast to LA which is present at substantial concentrations in most cellular lipids (membrane phospholipids plus neutral lipids including triglyceride) throughout various cells and tissues, LNA usually does not accumulate to particularly high concentrations in cellular tissue lipids even when consumed at substantial dietary levels. This is partly due to the extensive beta-oxidation of LNA \textit{in vivo}. The similar result is obtained in \textit{P. paradiseus} and \textit{P. pangasius} where LA contents are always lesser than LNA content in each lipid class. The presence of other members of \(\omega_3\) family such as 20:4\(\omega_3\), 20:5\(\omega_3\), 22:5\(\omega_3\), 22:6\(\omega_3\) in all cases perhaps supports evidence for efficient metabolic conversion of
LNA to its longer-chain products via the various desaturation/elongation reactions. Figure 22 also indicates the potential for DHA to undergo peroxisomal-mediated retroconversion to EPA.

The γ-Linolenic acid or 18:3ω6 (cis-6, cis-9, cis-12-octadecatrienoic acid), an important intermediate in the biosynthesis of arachidonic acid, occurs in only minor amounts in animal tissues. In S. phasa and G. chapra this acid is not found and in P. paradiseus and P. pangasius it is present.

Among ω3 fatty acids 5, 8, 11-Eicosatrienoic acid (20:3ω3) is normally a minor component of animal lipids but can assume significance in the complex fluids of animals deficient in essential fatty acids. In S. phasa this acid is found both in NL and PL but in G. chapra, it is only found in NL. In P. paradiseus and P. pangasius it is present in TL, NL, and GL but not in PL.

Arachidonic acid (cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid or 20:4ω6) is the most important of the linoleic acid metabolites and is a major constituent of the complex lipids of animal tissues. It is one of the principal precursors of a highly important series of hormone-like compounds known as prostaglandins (Ramwell et al., 1968) as shown in Fig. 22. These compounds have profound pharmacological activity and are currently the subject of intensive study; it now appears possible that there is a connection between certain of the symptoms of EFA deficiency and the presence or absence of prostaglandins (Christie, 2003). It should be noted that arachidonic acid has been regarded with some suspicion with respect to risks in cardiology (Weber, 1989), but together with DHA is an "essential" fatty acid in its own right at all times (Wander and Patton, 1991), as well as in pregnancy and infant nutrition (Ghebremeskel et al., 2000). The levels of 20:4ω6 are very low in a typical coldwater marine fish (Bhuiyan et al., 1986), but high in coastal-shelf tropical marine fish (Sinclair et al., 1983) as well as in a freshwater species Callichrous pabda that is also a popular food fish in India (Lakshmanan et al., 1999).

Fatty acid deficiency in fish species is indicated by the presence of 20:3ω9 acid (Watanabe, 1982). These fishes were collected from estuary where natural foods were available in abundance. Thus, the absence of eicosatrienoic acid in these fishes indicates that they are not suffering from fatty acid deficiency.

EPA (5, 8, 11, 14, 17- eicosapentaenoic acid) and DHA (4, 7, 10, 13, 16, 19-docosahexaenoic acid), in particular, are found in marine animal tissues as major components
of the complex lipids and they are also found in large amounts in fish oils. Buzzi et al. (1997a) provide evidence that the hepatopancreas of northern pike (*Esox lucius*) can convert alpha-linolenic acid to EPA and DHA. Such conversion is very essential for maintenance of fish physiology (Arts et al. 2001; March 1993). The presence of low amounts of 20:4ω3 and much higher levels of 20:5ω3 and 22:6ω3 in all lipid fractions of all the fishes under study suggests that they can convert 20:4ω3 to 20:5ω3 to 22:6ω3.

The ω3 PUFA are essential for the normal development of embryos, larvae, and the nervous system, and for the proper functioning of the sense organs of marine and freshwater fishes. The ω3 PUFA, also plays an important role in adaptation to changed environmental conditions and immunity to infections and parasitic diseases (Shulman and Love, 1999; Sargent et al., 1999).

There are many interacting external (temperature, salinity, food availability) and internal factors, including species, sex, physiological status (gonad maturity, health condition, age, etc.) that determine and affect the PUFA content of aquatic organism. Among them one of the main factors is diet. EPA, some DHA, and shorter-chain C16 and C18 ω3 PUFA are produced by microalgae, macroalgae and some bacteria (Lewis et al., 1999). Fishes take up the ω3 PUFA from their food, as essential nutritional components, which they cannot synthesize de novo. Along with simplified food chain, the animals can perform limited chain elongation and desaturation of the dietary ω3 PUFA. For this reason, the herbivores (e.g., abalone, oysters, mussels) and low-order carnivores (e.g., crustaceans) tend to contain more EPA and less DHA than high-order carnivores, which in turn contain less EPA than DHA (e.g., tuna, mackerel, shark, squid, octopus) (Dunstan et al., 1996, 1999).

Marine plankton contains more long-chain ω3PUFA than freshwater plankton. This is regarded as the major reason for the basic difference in the composition of fatty acid between marine and freshwater fishes. Most freshwater fishes and some marine species have active elongase and desaturase systems, allowing a rapid conversion to DHA and AA from shorter-chain ω3 and ω6 FA, respectively (Sargent, 1995). As opposed to freshwater fish, marine species cannot convert C-18 to C-20ω6 PUFA; thus, AA is also an essential FA in marine fish (Sargent et al., 1999).
4.3.2. THE TRANSPORT, BIOSYNTHESIS AND IMPORTANCE OF FATTY ACID CLASSES IN FISH PHYSIOLOGY

4.3.2.1. INTRACELLULAR TRANSPORT

The intracellular transport of free fatty acids in mammals is facilitated by specific low molecular weight tissue specific fatty acid binding proteins (FABPS) which have been characterized extensively in mammals (Veerkamp and Maatman, 1995). Several FABPS have been described from fish tissues, including elasmobranch livers (Bass et al., 1991; Baba et al., 1999; Cordoba et al., 1999). Muscle FABP has also been identified and characterized in Atlantic salmon (Torstensen, 2000). Recently, the molecular cloning and characterization of cDNAs for the FABPS from trout heart and zebrafish intestine, liver, and brain have been reported (Ando et al., 1998; Andre et al., 2000; Denovan-Wright et al., 2000a, b). The zebrafish intestine FABP was strongly expressed in the anterior intestine of larvae and expression correlated with the intracellular storage of lipid droplets in the enterocyte and synthesis of VLDL particles (Andre et al., 2000).

4.3.2.2. BIOSYNTHESIS OF DIFFERENT CLASSES OF FATTY ACIDS

Marine fish naturally consume diets rich in lipid, for example, capelin and their predators do not biosynthesize fatty acids de novo to any significant extent. Rather, the large lipid depots of these fishes frequently accumulate will be derived largely not exclusively from dietary lipid. The situation with freshwater fish may be different since lipid-rich preys are much less common in fresh water than in the sea (Tocher, 2003).

The key pathway in lipogenesis is catalyzed by the cytosolic fatty acid synthetase (FAS) multienzyme complex that occurs and has been characterized in fish (Sargent et al., 1989) using carbon source from acetyl-CoA formed in mitochondria from the oxidative decarboxylation of pyruvate (carbohydrate source) or the oxidative degradation of some amino acids (protein source).

4.3.2.2.1. SATURATED FATTY ACIDS (SFA)

The main products of FAS are the saturated fatty acids 16:0 (palmitic acid) and 18:0 (stearic acid), which can be biosynthesized de novo by all known organisms, including fish (Sargent et al., 1989) although a range of chain lengths from C12 to C24 can be found. Eight molecules of two-carbon acetyl units are required for the biosynthesis of 16:0 with one acetyl-CoA unit sewing as a primer and the further seven acetyl units being carboxylated by acetyl-CoA carboxylase to malonyl-CoA before being combined via FAS in a series of
sequential condensation steps requiring NADPH (Henderson and Sargent, 1985). However, phosphoglycerides that constitute animal cell membranes seldom contain significant amounts of saturated fatty acids other than 16:0, 18:0, and to a lesser extent 20:0, this restriction reflecting the relatively invariant geometry (width) of the phosphoglyceride-rich bilayers.

4.3.2.2. MONOUNSATURATED FATTY ACIDS (MUFA)

Monounsaturated fatty acids also occur naturally in chain lengths from about C14 to C24 but, although they are characterized by having a single unsaturated bond, the position of the ethylenic bond within the carbon chain can vary even within a specific chain length, so that there are considerably more species of monounsaturated fatty acids than saturated fatty acids.

Fish are capable of desaturating 16:0 and 18:0 to yield, respectively, 16:1ω7 (palmitoleic acid) and 18:1ω9 (oleic acid). Desaturation of fatty acids in fish, like all animals, takes place in the endoplasmic reticulum of cells of particular tissues via an aerobic process utilizing CoA-linked substrates and requiring NAD(P)H and O2, catalyzed by multi-component systems comprising NAD(P)H-cytochrome b5 reductase, cytochrome b5, and terminal desaturase enzymes (Brenner, 1974). This reaction is of particular physiological importance in that the monounsaturated products formed (16:1ω7 and 18:1ω9) have markedly lower melting points (phase transition temperatures) than their saturated precursors (16:0 and 18:0). Hence, Δ9 fatty acid desaturase provides a means of regulating the viscosity of cell membranes by altering the phase transition temperatures of the fatty acids in their constituent phosphoglycerides. The stearoyl Δ9 fatty acid desaturase activity has been particularly well characterized and its gene cloned in several animal species, including common carp (Tiku et al., 1996) and grass carp (Chang et al., 2001).

4.3.2.2.3. POLYUNSATURATED FATTY ACIDS

Marine organisms, especially algae, can contain a plethora of PUFA of chain lengths C16 (with 2-4 ethylenic bonds), C18 (with 2-5 ethylenic bonds), C20 (with 2-5 ethylenic bonds) and C22 (with 2-6 ethylenic bonds) (Sargent et al., 1995). These PUFA are generally of the ω3 series and in the case of C16 PUFA, the ω1, ω4 and ω7 series also occur (Ackman, 1989). However, in fish the main PUFA to be considered are 20:4ω6 and its metabolic precursor 18:2ω6 together with 20:5ω3 and 22:6ω3 and their metabolic precursor 18:3ω3.

All vertebrates, including fish, lack Δ12 and Δ15 ω3 desaturases and so cannot form 18:2ω6 and 18:3ω3 from 18:1ω9. Therefore, 18:2ω6 and 18:3ω3 are essential fatty acids in
the diets of vertebrates. These dietary essential fatty acids can be desaturated further and elongated to form the physiologically essential C20 and C22 PUFA, 20:4ω6, 20:5ω3, and 22:6ω3. The degree to which an animal can perform these conversions is dependent on the relative activities of fatty acid elongases and desaturases, such as ∆6 and ∆5, in their tissues, and these activities in turn are dependent on the extent to which the species can or cannot readily obtain the end product 20:4ω6, 20:5ω3, and 22:6ω3 fatty acids preformed from their natural diets. It is now established that the pathway biosynthesizing 20:5ω3 and 22:6ω3 from 18:5ω3 is present in rainbow trout (Buzzi et al., 1996, 1997). The major features of the pathway are summarized in figure 22. It is seen that, the affinity of the enzymes, especially the desaturases, is higher for the ω3 than for the ω6 series. The insertion of the last, ∆4, ethylenic bond in 22:6ω3 does not occur through direct ∆4 desaturation of its immediate precursor 22:5ω3. Rather, 22:5ω3 is chain elongated to 24:5ω3, which is then converted by ∆6 desaturation to 24:6ω3, which is then converted by a chain-shortening reaction in the peroxisomes to 22:6ω3. Experiment to study the conversion of the 18:3ω3 to 20:5ω3 and then to 22:6ω3 by radioisotopes administered feeding in vivo supported that conversion occurs in many freshwater species of fish but poorly in marine fish studied (Sargent et al., 1989, 1995, 2002). It reflects the fact that 20:5ω3 and 22:6ω3 are very abundant in the marine environment, originating mainly in diatoms and flagellates, respectively, at the base of the food web, where they are transmitted intact via zooplankton to fish (Ackman, 1989). In contrast, the natural prey of many freshwater fish, particularly their invertebrate prey, is not rich in 22:6ω3, being rich instead in 18:2ω6, 18:3ω3, and to a lesser extent 20:5ω3. Thus, marine fish remained in an environment where such conversion is not necessary like the freshwater fishes.

4.3.2.3. IMPORTANCE OF FATTY ACIDS IN FISH

4.3.2.3.1. ENERGY PRODUCTION BY FATTY ACID CATABOLISM

The combination of 14:0 and 16:0 help the fish to maintain their body temperature regulation via respiration. The catabolism of fatty acids occurs in the mitochondria (and peroxisomes) via a completely different set of enzymes by the process is termed β-oxidation to produce acetyl-CoA and NADH. The acetyl-CoA can then be metabolized via the tricarboxylic cycle to produce more NADH. The NADH produced from the oxidation of fatty acids can then provide metabolic energy in the form of ATP through the process of oxidative phosphorylation. The available evidence is consistent with all saturated and monounsaturated
fatty acids, including 22:1ω11, being readily catabolized by mitochondrial β-oxidation in fish (Sargent et al., 1989). However, β-oxidation of PUFA is variable between different PUFA molecules and can be more complicated. However, relatively high levels of peroxisomal β-oxidation were observed in the red muscle of Atlantic salmon (Froyland et al., 2000). Peroxisomal β-oxidation may account for significant amounts (up to 50%) of total hepatic β-oxidation under certain conditions such as in Antarctic fish (Crockett and Sidell, 1993).

Current knowledge of the relative importance of individual fatty acids in energy provision is that the fatty acids are the predominant sources of potential metabolic energy include 16:0 and 18:1ω9, the 20:1ω9 and 22:1ω11 that are particularly abundant in the so-called northern fish oils, and the ω3 HUFA 20:5ω3 and 22:6ω3. Undoubtedly, 16:0, 18:1ω9, 20:1ω9, and 22:1ω11 are heavily catabolized for energy in fish because they are all consumed in large amounts during the growth of farmed fish species such as the salmonids, and, specifically, during formation of roe by female fish (Henderson et al., 1984a, b; Henderson and Almater, 1989). In Antarctic fish tissues, monoeneoic fatty acids were preferentially oxidized for energy compared with long-chain saturated fatty acids (Sidell et al., 1995). The use of medium-chain triacylglycerols (MCT) containing 6:0, 8:0, 10:0, and 12:0 is beneficial as alternative sources of energy and may also have an important effect in lowering body fat levels. Studies in Atlantic salmon showed that 8:0 and 10:0 were highly digestible (99.6% and 96.7%, respectively) and were mainly absorbed in the pyloric region, but they appeared to reduce pyloric absorption of other fatty acids (Roesjoe et al., 2000).

4.3.2.3.2. ROLE OF FATTY ACID IN FISH REPRODUCTION AND EMBRYOGENESIS

Fatty acids are not only the major source of metabolic energy in fish for growth from the egg to the adult fish (Tocher et al., 1985a, b), they also are the major source of metabolic energy for reproduction (Henderson et al., 1984a, b; Sargent et al., 1989). For successful reproduction, the stored oil must support not only the immediate energy requirements of the parent fish but also the future requirements of the progeny. The total lipid of eggs from most fish studied is generally rich in ω3 PUFA (Sargent et al., 1989). Egg phosphoglycerides, like phosphoglycerides in other tissues, are generally higher in PUFA than neutral lipids so that egg PUFA levels partly reflect the relative amounts of polar and neutral lipid. Thus, marine eggs tend to have higher ω3 PUFA levels than freshwater species (Rainuzzo, 1993; Wiegand, 1996a). In contrast, the eggs of most freshwater fish contain higher levels of ω6 PUFA, particularly 20:4ω6 and 18:2ω6, than marine fish eggs (Anderson et al., 1990; Wiegand,
The utilization of lipids and fatty acids during embryonic and early larval development varies considerably between fish species. Lipids were utilized as an energy source mainly after hatching in goldfish (Wiegand, 1996b), sturgeon (Gershanovich, 1991), red sea bream, and winter flounder (Sargent et al., 1989), whereas in pike (Desvilettes et al., 1997), striped bass (Chu and Ozkizilcik, 1995), Atlantic herring and cod, lipids were utilized during both embryogenesis and early larval development (Tocher et al., 1985a; Fraser et al., 1988).

The DHA plays an important reproductive role in females. It is transferred from muscles to the liver and gonad products and influences the survival of eggs and larvae. The reproductive capacity of the females can be deduced from the level of DHA in their TG (Shulman and Love, 1999). DHA also plays an important role in adaptive processes (temperature, salinity, and oxygen adaptations), as well as in the motoric and social behavior of fish (Shulman and Love, 1999). There is a correlation between the fish DHA content and mobility of salt and freshwater fish (Reinhardt and van Vleet, 1986; Shulman and Love, 1999). The lipid in vitellogenin is predominantly phospholipid (about 65 to 70% of total lipid) and is rich in ω3 PUFA, particularly 22:6ω3, which accounts for 20% of total fatty acids in trout vitellogenin (Leger et al., 1981). In fish, dietary deficiency of 22:6ω3 resulted in larval herring having an impaired ability to capture prey at natural light intensities (Bell et al., 1995) and impaired schooling behavior in yellow-tail (Masuda et al., 1998; Ishizaki et al., 2001) and Pacific threadfin (Polydactylus sexfilis) (Masuda et al., 2001). These recent studies imply a critical role for 22:6ω3 in the functioning of neural tissue (brain and eye) in fish and also demonstrate the importance of dietary 22:6ω3 in marine fish.

4.3.2.3.3. ROLE OF FATTY ACID IN EICOSANOIDs PRODUCTION IN FISH

The two main enzymes involved in eicosanoids production are cyclooxygenase that produces cyclic oxygenated derivatives or prostanoids, including prostaglandins (PG), prostacyclins (PGI) and thromboxanes (TX), and lipoxygenases that produce linear oxygenated derivatives, including hydroperoxy- and hydroxy fatty acids, leukotrienes (LT), and lipoxins (LX). Collectively, these fatty acid derivatives are termed eicosanoids, so named because they are derived primarily from the C20 PUFA 20:3ω6, 20:4ω6, and 20:5ω3.

The eicosanoids are autocrines, that is, hormone-like compounds produced by cells to act in their immediate vicinity with a short half-life. Virtually every tissue in the body produces eicosanoids, and they have a wide range of physiological actions, for example, in
blood clotting, the immune response, the inflammatory response, cardiovascular tone, renal function, neural function, and reproduction. Eicosanoids have been found in a large range of freshwater and marine fish, and virtually every tissue so far studied has shown cyclooxygenase and/or lipoxygenase activity with gills generally being the most active. Fish produce the same range of eicosanoids as in mammals with the prostanoids, PGE, PGF, and PGD, and TXB and 6-keto-PGF\textsubscript{1α}, the respective stable metabolites of TXA and PGI\textsubscript{2}, all being reported in fish (Tocher, 2003).

It is seen that 20:4\textsubscript{ω6} is the chief precursor of the eicosanoids, generating 2-series prostanoids and 4-series leukotrienes. However, 20:5\textsubscript{ω3} competes with 20:4\textsubscript{ω6} in eicosanoid production, and is itself converted to 3-series prostanoids and 5-series leukotrienes, which are generally less biologically active than the corresponding 2-series prostanoids and 4-series leukotrienes produced from 20:4\textsubscript{ω6}. Thus, eicosanoid actions are determined by the ratio of 20:4\textsubscript{ω6}:20:5\textsubscript{ω3} in cellular membranes, this in turn being determined by the dietary intake of \textit{ω}6 and \textit{ω}3 PUFA as illustrated in figure 23.

**4.3.3. HEALTH BENEFITS OF DIFFERENT FATTY ACID CLASSES ON HUMAN PHYSIOLOGY**

The 18 carbon α-linolenic acid has not been shown to have the same cardiovascular benefits as DHA or EPA. Currently there are many products on the market which claim to contain health promoting 'omega 3', but contain only α-linolenic acid (ALA), not EPA or DHA. These products contain mainly higher plant oils which have to be converted by the body to create DHA and therefore considered less efficient. DHA and EPA are made by microalgae in seawater which are then consumed by fish and accumulate to high levels in their internal organs. Thus, the artificial supplementation cannot be a healthy alternative of evolutionary fixed route of fish consumption by human.

In a study of nearly 9,000 pregnant women, researchers found women who ate fish once a week during their first trimester had 3.6 times less risk of low birth weight and premature birth than those who ate no fish. Low consumption of fish was a strong risk factor for preterm delivery and low birth weight (Olsen and Secher, 2002; Ghebremeskel \textit{et al.}, 2000). Fish oil stimulates blood circulation, increases the breakdown of fibrin, a compound involved in clot and scar formation, and additionally has been shown to reduce blood pressure (Morris \textit{et al.}, 1993; Mori \textit{et al.}, 1994). There is strong scientific evidence that \textit{ω}3 fatty acids reduce blood triglyceride levels (Roche and Gibney, 1996; Harris, 1997; Sanders
et al., 1997) and regular intake reduces the risk of secondary and primary heart attack (Burr et al., 1994; Stone, 1996; Bucher et al., 2002;).

Several studies report possible anti-cancer effects of ω3 fatty acids (particularly breast, colon and prostate cancer) (De Deckere, 1998). ω3 fatty acids reduced prostate tumor growth, slowed histopathological progression, and increased survival. Among ω3 fatty acids neither long-chain nor short-chain forms were consistently associated with breast cancer risk. High levels of docosahexaenoic acid, however, the most abundant ω3 PUFA in erythrocyte membranes, were associated with a reduced risk of breast cancer. The ω3 fatty acids are known to have membrane enhancing capabilities in brain cells. One medical explanation is that ω3 fatty acids play a role in the fortification of the myelin sheaths. There is considerable evidence supporting the hypothesis that high incidences of cardiovascular and inflammatory conditions, and some cancers, in developed societies are associated with an excessive dietary intake of 18:2ω6 relative to 18:3ω3, which generates high levels of 20:4ω6 in cells and consequently pathological levels of eicosanoids (Okuyama et al., 1997).

Dietary supplementation with 20:5ω3, as fish or fish oil, can be beneficial by damping down excess eicosanoid production from 20:4ω6 (Anon, 1999). Thus, although both 20:4ω6 and 20:5ω3 serve as eicosanoid precursors it has been established in fish that 20:5ω3 and 20:3ω6 (dihomo-γ-linolenic acid) which can also serve as a substrate for fish cyclooxygenase enzymes, competitively depress the production of eicosanoids from 20:4ω6 (Bell et al., 1994). Therefore, in fish, as in mammals, eicosanoid production is influenced by the cellular ratio of 20:4ω6: 20:5ω3, although the optimal ratio of 20:4ω6: 20:5ω3 for eicosanoid production is probably lower in fish than in mammals. Irrespective of details, an imbalanced ratio of 20:4ω6: 20:5ω3 appears to be as damaging in fish as in mammals.

The distribution of SFA, MUFA, and PUFA content within different lipid fractions depends upon age, breeding season, activity of elongase and desaturase enzymes, etc. Thus within the individuals of the same species it may vary (Zenebe, 1998). SFA, MUFA, and PUFA content of some selected commercial food fish of India comparing data from various workers is presented in table 9 and depicted in the figure 24. It was seen that fatty acids from freshwater fish were less saturated (25.11-44.5%) than marine fish (49.2- 69.43%) but the range of MUFA content of fresh water fish is narrower (20-34.1%) than that of marine fish (12-42.53%). The maximum PUFA content in the freshwater fish is in cyprinus carp and in the marine fish pomfret.
The ratio of ω3 PUFA to ω6 PUFA can be used to facilitate identification of high ω3 PUFA foodstuffs. In general, the ω3/ω6 ratio is higher for marine foodstuffs (figure 19). Freshwater fish contain higher proportions of ω6 acids and lower ω3, allowing differentiation between freshwater and marine species based on the ratio of these two types of PUFA (Ackman, 1994b; Sargent, 1995; Nichols et al., 2001b). High occurrence of ω3 in marine fish can be analyzed in view of first line innate immune defense mechanism in absence of mucus as well as epidermis to minimize the ion absorption process in sea water. Only a few studies have assessed changes in lipolytic capacity of gill cells during osmotic acclimatization addressing an increased capacity by directly affecting electrolyte transport and indirectly through prostaglandins (Ackman and Eaton, 1966; Ackman, 1967; Li and Yamada, 1992). It has been suggested that the ω3/ω6 ratio of 1:1 to 1:5 would contribute to a healthy human diet (Osman et al., 2001) and WHO recommendation is the daily ratio of ω3/ω6 in total human diet should be more than 1.5 (Vujkovic et al., 1999).

The ratio ω3/ω6 was much lower in cultured sweet smelt than in wild individuals of this species (Jeong et al., 2000). The ω3/ω6 ratio in muscle lipids of common farmed fish species (rainbow trout, European catfish, pike, grass carp, ide, and carp) varied front 0.74 (ide) to 3.45 (pike) (Bieniarz et al., 2000). The ω3/ω6 ratio is lower in farmed fish due to the composition of the feeds they are fed, that is, typically high vegetable oil plant product diets rich in 18:2ω6 and conditions under which they are kept (i.e., lower mobility, reduced need to forage) (Shulman and Love, 1999). On the contrary, in A. mola, the ω3/ω6 ratio is ranging between 6.9-15.0, though it is a minor freshwater carp living mainly on natural feeds (Dey et al., 2015). The fish-based ω3 PUFA consists mainly of EPA and DHA. Much smaller amounts of docosapentaenoic acid (DPA, 22:5ω3) are found in fish and fish oils although they represent up to 5% of the fatty acids in certain marine mammal sources such as seal oils.

Table 9 shows that excepting L. bata, C. mrigala, N. saldado, T. ilisha, G. chapra, in all other fish DHA was found in higher levels than EPA. These data confirmed earlier observations of Gruger et al. (1964) and Gunstone et al. (1978). As it can be seen from the Table-9 freshwater fish presented very low amount of 20:5 and 22:6ω3 fatty acids in their flesh when compared to marine fish.

Essential fatty acids give rise to prostaglandins, thromboxanes, lipoxins and leukotrienes in the body. Essential fatty acids and other ω3 and ω6 polyenoic acids are used in PL synthesis and are incorporated particularly as the 2-acyl (beta-acyl) groups in
phospholipids. As constituents of membrane phospholipids, essential fatty acids and other polyenoic acids play important roles in maintaining structural integrity and fluidity of membranes. Arachidonic acid, for example, constitutes up to 15% of the fatty acids of membrane phospholipids; DHA, formed from linolenate or obtained from dietary fish oils occurs almost in each membrane phospholipid molecule of outer segments of retinal rods. By participating in PL formation they promote fat mobilization and prevent fatty liver. DHA, formed from linolenic acid, is required for the development of brain and retina. Low blood DHA levels have been correlated with the occurrence of the disease retinitis pigmentosa.

PUFA help to lower the serum LDL-cholesterol because the cholesteryl esters of essential fatty acids and other polyenoic acid formed from them are more rapidly removed from blood and metabolized than those of saturated fatty acids. EPA and DHA seem to alter the metabolism of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and intercellular adhesion molecule-1 (ICAM-1) (Eritsland et al., 1996, Abe et al., 1998).

Incorporation of ω3 type PUFA into cardiovascular tissue membranes occurs readily, mainly at the expense of PUFA of the ω6 type (18:2 and 20:4). The benefits of ω3 fatty acids against thrombogenic cardiovascular risk factors are achieved via a favourable eicosanoid profile. Compared to the prostaglandins and thromboxanes of the 2-series (PGL2 and TX2) derived from membrane arachidonic acid (20:4ω6), ω3 PUFA result in the production of PGL3 and TX3 (Weber, 1989). EPA inhibits formation of TX2 and is a precursor of the series-5 leukotrienes, compound with substantially lower physiological activities than their arachidonate-derived (series-4) counterparts. This suggests that a diet containing marine lipids should decrease the extent of prostaglandin and leukotrine mediated inflammatory response (Voet and Voet, 1995). In addition, the ω3 series of eicosanoids have different antithrombogenic potencies in comparison to those derived from ω6 PUFA. Thus, PGL3 is equipotent to PGL2 in antithrombotic actions, while TX3 possesses only weak biological activity. Thus the risk for ischemic heart disease is reduced (Fitzgerald et al., 1989).
4.4. QUALITATIVE AND QUANTITATIVE ASSESSMENT OF FISH FAT

The benefits of consuming fish depend not only on the amounts of lipid and fatty acids present in the fish muscle but also on the proportionate fraction of a particular class and how much fish should be consumed to maintain better health. The qualitative assessment of fish fat is obtained by atherogenic index (AI) and thrombogenic index (TI) and the quantitative assessment of fish fat is derived by calculating the serving frequency of fish per week.

4.4.1. QUALITATIVE ASSESSMENT OF FISH FAT: CALCULATION OF AI AND TI

The AI and TI were calculated according to Ulbricht and Southgate (1991). The fat quality of fish species is evaluated by the AI which is the ability to reduce the blood lipid content; and the TI which is the ability to reduce the platelet activity (Ulbricht and Southgate, 1991).

Among the PUFA’s are known components with anti-atherogenic action like LA (18:2ω6) belonging to the ω6 PUFA’s class, and in the more important ω3 PUFA’s class, components such as LNA (18:3ω3), EPA (20:5ω3) and DHA (22:6ω3) which are appreciated for their anti-thrombogenic effect. On the contrary, among the saturated fatty acids (SFAs), lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0), are recognized as health risk factors. Diets rich in monounsaturated fatty acids (MUFAs) resulted very efficient in reducing the coronary diseases risk. Indeed MUFA have been recognized as beneficial as the PUFA’s of ω3 class for human health because of their effect in lowering blood cholesterol.

Index of atherogenicity is indicating the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated, the former being considered pro-atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory system), and the latter anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids), thereby preventing the appearance of micro- and macro- coronary diseases.

Index of thrombogenicity is showing the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (MUFAs, ω6 and ω3 PUFAs).

In the present study it is evident from the data that both the AI and the TI indices for all the fishes are present in fair amounts. The TI values of the TL are ranging between 0.48-0.88 for these fishes under study. These low TI values (<1), for all the fish flesh suggest a
high anti-thrombogenic quality of fish meat in contrast to the TI values of beef (1), lamb meat (1.4) and milk-based products (2.1) (Amerio et al., 1996). These results are a clue to the fact that fish do not die from myocardial infarction. Interestingly, diet with low AI and TI values lowers TG levels particularly in patients with hypertriglyceridemia. This effect is not seen with plant sources of ω3 PUFA (Kestin et al., 1990).

4.4.2. QUANTITATIVE ASSESSMENT OF FISH FAT: CALCULATION OF SERVING FREQUENCY PER WEEK

For patients with documented CHD, the American Heart Association guidelines advise 900-1000 mg/day of EPA/DHA combined (Krauss et al., 2000; Kris-Etherton et al., 2002). They have indicated that for secondary prevention, this target would require one fatty fish meal per day or alternatively supplementation with EPA/DHA from fish oil sources. As presented in figure 25 the 900 mg/day target for EPA/DHA could require 3-286 servings of fish/week depending upon the source/type chosen. From that angle of view S. phasa requires 9 servings, G. chapra requires only 4 servings, P. paradiseus requires 22 servings and P. pangasius requires 46 servings/week to meet AHA guidelines for the patients with documented CHD but for normal individuals without documented CHD, AHA recommends to eat a variety of fish (preferably oily) at least twice a week. The proposal for adequate daily intake of EPA+DHA for adults is to be at least 220 mg/day; and from that point of view these fishes under study will require much less servings. The correlation between the occurrence of various lipids and fatty acid classes from the muscles of the fishes under present study, the importance of respective lipids and fatty acids in fish biology and human nutrition is summarized in figure 26.

Depending on the habitat preferences, availability of lipid rich food materials, etc. these fishes under study are showing a little inequity from some aspects of their ω3/ω6 ratio, serving frequency, etc. Since S. phasa and G. chapra obtain lipid rich marine food sources, their TL contents, ω3 contents, ω3/ω6 ratio resembles with the stenohaline sea fishes (Majumder et al., 2015). On the contrary, the adults of P. paradiseus and P. pangasius feed mainly on mollusks and crustaceans available in freshwater spawning habitat which results oppositely to their TL contents, ω3 contents, ω3/ω6 ratio akin to freshwater fishes. P. pangasius although requires high servings but it is also known to withstand at low oxygen levels. For extensive feeding on molluscan shells, P. pangasius can be safely utilized as a biological control agent, consequently preventing infestation of disease, such as
schistosomiasis for which mollusks are known vectors. This fish would not only control the proliferation of mollusks but also add to the productivity in such water bodies. David (1963) has reported that, in the food habits at all stages of its life, the fish is compatible with carps; hence it can be a useful addition in carp polyculture.

It is observed from the results of the present study that these edible marine fishes follow similar pattern of lipid and fatty acid distribution and contain sufficient amount of ω3 and ω6 fatty acids which was anticipated in the null hypothesis. Hence the null hypothesis is proved and can be said that these fishes can maintain better cardiac health upon consumption by human. Being comparatively cheap, tasty, nutritious as well as preventive and therapeutic qualities against chronic heart diseases, all of them can be a part of our staple diet.