6. **Summary**
A major nutrition-related problem in India is chronic under-nutrition associated with low fat intake. Under-nutrition starts even at the time of conception and a large number of infants are born with low birth weight. Recent evidences indicate that low birth weight babies are at a greater risk of developing CHD in later life as compared to those born with normal birth weight. PUFA have both curative and preventive effects on cardiovascular diseases and as well as many other diseases such as asthma, hypertension, diabetes, cancers, etc. It also has immense effects on neurodevelopment in infants and it delays the process of brain aging. Thus optimal PUFA intake is essential at all stages of life – conception, growth & development. AHA has recommended taking 900-1000 mg of PUFA per day can maintain better cardiac health. On the basis of that a serving frequency of fish per week is formulated taking the fish as a source of PUFA.

Compared to red meat fish flesh is easily digestible and provides energy, high level proteins and significant amount of PUFA. The value of fish as a source of long chain ω3 fatty acid primarily depends on the lipid content in the muscle. Being the chief site of lipid synthesis as well as of storage the liver of lean fish shows much higher level of lipid classes than those of muscles but usually the gut contents are discarded during processing of fish as food. Thus the muscle lipid becomes the chief source of fish lipid upon consumption by human.

It was a long practice to determine the nutritional values of Indian fishes from the aspects of lipids and fatty acids which are still being carried out. 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 reference about their nutritional quality particularly from the aspect of lipids and fatty acids. This scarcity of data does not lead to a beneficial conclusion on behalf of the consumption of these fishes. Considering this 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 four fish species namely *Setipinna phasa*, *Gudusia chapra*, *Polynemus paradiseus* and *Pangasius pangasius*. 2. To estimate the neutral lipid and phospholipid fractions. 3. To evaluate the distribution of different fatty acids among the various lipid classes. 4. To calculate ω3 and ω6 ratio and compare EPA and DHA in each of the fish. 5. To derive the atherogenic index (AI) and thrombogenic index (TI) and the serving frequency per week for all of the fishes.
The hypothesis of the present study is that as marine fish 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 ω3 and ω6 fatty acids.

Prior to the biochemical investigations almost equal sized adult fishes irrespective of their sexes were netted out from Bakkhali, South 24 parganas, West Bengal, India through local fisherman during monsoon. They were kept in -4°C and brought to the laboratory. Prior to lipid extraction, the fishes were de-scaled, the fins, heads and other visceral parts except liver were discarded. 100g of flesh was pooled from 10 fish samples of each species having almost equal length and weight. From this 100g flesh total lipids were extracted. A portion of the total lipid was subjected to Thin Layer Chromatography (TLC) using silica gel for lipid class separation. The NL components obtained from the samples were HC, WE, SE, ADAG, TG, and ST. Total phospholipids from the samples were classified into different classes viz., CL, PE, PC, PI, and SPH by one dimensional TLC using chloroform: methanol: 28% ammonia (65:25:5,v/v/v) with co-chromatography of a standard phospholipids mixture (PE, PC, PI, PS, and SPH), applied in a separate lane. The different classes of phospholipids were identified by suitable staining reagent viz. Molybdenum blue reagent for all glycerophosphatides, Dragendorff reagent for glycerophosphatidyl-choline and ninhydrin reagent for glycerophosphatidyl-ethanolamine. Fatty acid methyl esters (FAME) of TL, NL, GL and PL were prepared by transmethylation and pure methyl esters thus obtained were redissolved in n-hexane, sealed under a nitrogen stream and kept in freezer for GLC analysis. GLC of fatty acid methyl esters were done on a Chemito 1000 instrument, equipped with Flame Ionization Detector. Quantization was done by computer using specific Clarity Lite software.

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. The total lipid content in the fish is very vital to regulate various physiological functions like energy production, locomotion, reproduction, etc. which is determined by the homeostasis between lipid intake and expenditure.

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 phytoplankton. 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. 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 phytoplanktons. On the contrary, *S. phasa* and *G. chapra* obtain lipid rich marine food sources which results in high fat content in both of them.

Among the major fractions of the total lipid, NL was found to be predominant followed by PL and GL in all cases. The TL of *S. phasa* and the GL of *P. pangasius* are predominant over others. Among the major fractions of the NL, TG found to be the most predominant followed by ADAG in all of them. The amount of TG in *P. pangasius* and the amount of ADAG in *G. chapra* are little more than rests. By sequential esterification TG is synthesized from glycerol-3-phosphate and dietary fatty acids and stored for long term storage into mesenteric adipose tissue, white muscle, etc. TG can be used surplus to the available dietary energy source when energy requirement is high for reproduction, migration, etc.

Comparing the PL fraction, PC is found to be largest followed by CL in all the fish species. The PE and PI values of *G. chapra*, CL of *S. phasa*, PC of *P. paradiseus* and SPH content of *P. pangasius* are found to be high. Dietary PL is digested by pancreatic or intestinal phospholipase and then absorbed by intestinal mucosal cells in fish. PL can also be synthesized in the body of the fish to regulate membrane structure and function and embryonic development. Among the PL fractions the PC and PE are catabolized for energy production during embryogenesis. PC is also utilized for bile formation in fish.

Composition of twenty five fatty acids quantified by open tube gas-liquid chromatography from the body flesh of *S. phasa* is found. The amount of SFA was rampant in GL and PL. Among SFA palmitic acid, and among MUFA oleic acid, was predominant. DHA is the major PUFA in *S. phasa*. EPA is found to be the second largest PUFA in all lipid classes.
Twenty six fatty acids were quantified by open tube gas-liquid chromatography from the body flesh of *G. chapra*. The amount of SFA is rich in GL. Among SFA palmitic acid (16:0) is the predominant. Oleic acid is the most abundant MUFA and EPA is the major PUFA in this fish.

Total thirty one fatty acids were quantified by open tube gas-liquid chromatography from the body flesh of *P. paradiseus*. The amount of SFA is utmost in NL, where as MUFA is highest in GL. Among SFA, palmitic acid (16:0) is rich. Oleic acid is the most abundant MUFA and DHA is the major PUFA in this fish.

Altogether thirty fatty acids were quantified by open tube gas-liquid chromatography from the body flesh of *P. pangasius*. The amount of SFA is rich in NL, where palmitic acid (16:0) is the predominant one. Among MUFA, oleic acid is the most abundant and EPA is the major PUFA excepting PL in this fish.

Comparing the fatty acids of TL in these fish species, it is evident that the SFA content is high in *P. pangasius* where as in *S. phasa*, the MUFA content is greater but PUFA content is predominant in *G. chapra*. Similar results are arising in comparing the fatty acids of NL but comparing the fatty acids of GL, the SFA content is found to be rich in *S. phasa* where as in *P. paradiseus* the MUFA content is greater but PUFA content is predominant in *P. pangasius*. The SFA content of PL in *S. phasa* is maximum and PUFA is predominant in *G. chapra* than rests.

The ratio of ω3 PUFA to ω6 PUFA can be used to facilitate identification of high ω3 PUFA foodstuffs. It has been suggested by WHO that daily ratio of ω3/ω6 in total human diet should be more than 1.5. Since all the fish under study have good amount of ω3 fatty acid, they have a fair ω3/ω6 ratio. Comparing ω3 fatty acids, it is evident that the ω3 content is highest in PL followed almost by TL in all cases. Among them maximum ω3 fatty acids are present in *G. chapra*. The comparative occurrence of ω6 fatty acids reveals that ω6 is predominant in *P. pangasius* followed by *P. paradiseus* in their GL. It is evident from different studies that ω3/ω6 ratio is higher in the marine fishes in contrast to their freshwater counterparts. The ω6 fatty acids produce series 2 prostaglandins, series 2 thromboxanes and series 4 leukotritines but ω3 fatty acids produce series 3 prostaglandins, series 3 thromboxanes and series 5 leukotritines. Series 3 prostaglandins is equipotent to series 2 prostaglandins in antithrombotic actions, while series 3 thromboxanes possesses only weak biological activity. The series 2 thromboxanes have strong platelet aggeregation and thrombus formation ability.
than series 3 thromboxanes. Thus the risk for ischemic heart disease is comparatively less with ω3 rich food stuff. Again, series 5 leukotrienes have substantially lower physiological activities than their series 4 counterparts. This suggests that a diet containing marine lipids with more ω3 should decrease the extent of prostaglandin and leukotrine mediated inflammatory response.

The EPA and DHA contents in these fish are found to be predominant in PL. Excepting the GL, for each of the lipid fractions, it is evident that EPA contents of *G. chapra* are predominant over others. Comparing DHA content, it can be said that DHA content in PL of *P. paradiseus* are predominant over others.

The fat quality of these fish species were evaluated by the atherogenic index (Al) which is the ability to reduce the blood lipid content and the thrombogenic index (TI) which is the ability to reduce the platelet activity (Ulbricht and Southgate, 1991). In the present study it is evident from the data that both the Al and the TI indices for all the fishes are present in fair amount. These TI values 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).

For patients with documented CHD, the American Heart Association guidelines advise 900-1000 mg/day of EPA/DHA combined. 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 per week. For normal individuals without documented CHD, AHA recommends to eat a variety of (preferably oily) fish 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.

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