Major lipid classes and their fatty acid composition in the flesh of two edible marine food fishes from India

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Abstract

Objective: The present analysis investigated various lipid classes and their fatty acid composition from the body flesh of two commercially cheap marine fish species- Gangetic hairfin anchovy (Setipinna phasa) and khoira (Gudusia chapra) for the exploration of the nutritional quality to assess their usefulness for human consumption.

Scope: These two fishes are found in profusion but in spite of their importance in capture fisheries in Bay of Bengal, nutritional assessment of fat quality has not been done so far. This study evaluated the pattern of distribution and amount of principal lipids and their fatty acids by TLC (thin layer chromatography) and GLC (gas liquid chromatography) from the muscles of these two fishes to evaluate the health benefits for the consumers.

Major findings: Both in S. phasa and G. chapra, the total lipid (TL) contents are 12.51 and 10.72 respectively, for which they are considered as high fat fish. Among TL fractions, neutral lipid (NL) was predominant followed by phospholipid (PL) and glycolipid (GL). Within NL fractions, triacylglycerol (TG) was found to be prime followed by 1-O-Alkyl-2,3-diacylglycerol (ADAG) and in the PL fractions, Phosphatidylcholine (PC) was largest. Twenty five fatty acids from S. phasa and twenty six fatty acids of G. chapra were quantified. Among saturates, palmitic acid (16:0) and among monounsaturates, oleic acid (18:1) were abundant in both. In S. phasa DHA and in G. chapra EPA was the major polyunsaturated fatty acid (PUFA).

Conclusions: These two fish show the similar pattern of muscle lipid and fatty acid distribution as well as contain sufficient amount of ω3 and ω6 fatty acids which can maintain better cardiac health upon consumption by human.

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Key words: Fatty acid composition; GLC; Glycolipid; Marine fish; Neutral lipid; Phospholipid ; Total lipid.

1. Introduction

The unrevealed silence of marine world is blessed with diversified aquatic wealth including edible fish. The Hooghly estuarine system along the Indian coast of Bay of Bengal is one of the largest and most productive estuaries in India. It is located in West Bengal between latitude 21°31´N and 23°30´N and longitude 87°45´E and 88°45´E. The total length of the tidal Hooghly estuary is about 295 km. FAO species survey reports that 475 species of fish under 138 families existed in Bay of Bengal (1) most of which are still unutilized or poorly known for their nutritional quality. In addition to energy supplier, marine fish are consumed as a source of vitamins and polyunsaturated fatty acid (PUFA) (2) though the composition of fatty acid in the edible part of fish is affected by many factors, such as species, sex, sexual maturity, size, place of capture, water temperature, feeding and season (3, 4).

Fish is quite different from the other animal food sources because they provide low energy, high level proteins and significant amount of polyunsaturated fatty acids and as compared to red meat fish flesh is easily digestible because it contains long muscle fiber (5). The value of fish as a source of long chain ω3 fatty acid primarily depends on the lipid content and the region from which the fish are harvested, with the highest contents generally found in fat fish caught in colder region (6). The main characteristic difference in freshwater fish is the higher levels of C16 and C18 acids and the lower levels of C20, and C22 acids when compared to marine fish, and these differences are mainly
due to dietary fat (7, 8). Lipids and fatty acids play a significant role in membrane biochemistry and have direct effect on the membrane-mediated process in animals such as osmoregulation, nutrient assimilation and transport (9).

Initially the nutritional value of Indian fishes was investigated by Saha and Ghosh (10, 11) and now many studies (8, 12, 13, 14 and 15) are being carried out about the nutritional quality of the edible marine fish muscles which forms part of the staple diet of a large percentage of Indian population. Observations on Eskimos in 1970s sparked great interest in ω3 PUFA research and today we know that both ω6 and ω3 PUFA have curative and preventive effects on cardiovascular diseases, asthma, hypertension, diabetes, cancers, brain aging, neurodevelopment in infants’ and fat glycemic control (16, 17). In addition, the benefits of ω3 PUFA are associated with the synthesis of eicosanoids such as prostaglandins, thromboxanes, and leukotrienes (18). These ω3 fatty acids can be divided into three main categories: Alpha-linolenic acid (ALA, C18:3ω3), Eicosapentaenoic acid (EPA, C20:5ω3), and Docosahexaenoic acid (DHA, C22:6ω3). Results of clinical and epidemiological research suggest that EPA and DHA, found only in fish and sea foods, have extremely beneficial properties for the prevention of human coronary artery disease (19) but both EPA and DHA cannot be synthesized in the human body and thus need dietary intake (20). EPA and DHA are typically found in marine fish and originate from the phytoplankton and seaweed that are part of their food chain (2, 7). In addition to EPA, DHA and ALA, all the studies that have been done so far on the composition of lipids in fishes have shown that there are a few important major fatty acids like myristic (C14:0), palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1ω7), oleic (C18:1ω9), linoleic (C18:2ω6), α-linolenic (C18:3ω3) and arachidonic acid (C20:4ω6) and a few minor ones that are present in trace amounts (21, 22, 23, 24, and 25).

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 on their nutritional quality particularly from the aspect of lipid. This scarcity of data does not lead to a beneficial conclusion on behalf of the consumption of this fish. Considering this lacunae, the present study is aimed to evaluate the total lipid and relative proportions of various lipid classes from the body flesh of two marine fishes- Setipinna phasa and Gudusia chapra as well as, estimate the neutral lipid and phospholipid fractions and distribution of different fatty acids among the various lipid classes. From the derived data the ω3/ω6 ratio, atherogenic index (AI), and thrombogenic index (TI) are also calculated. For the patients with documented CHD, this study aims to evaluate the serving frequency of these fishes per week based on the prescribed guidelines recommended by AHA. 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.

2. Materials and Methods

Setipinna phasa (Hamilton 1822), the hairfin anchovies, is a brackish water, pelagic, amphidromous fish of the family- Engraulidae, under the order- Clupeiformes, under the class- Actinopterygii. This fish is available in Indian fresh and brackish waters (Ganges system, from Diamond Harbor on the Hooghly to as far up as Allahabad on the Ganges, perhaps further; also rivers and estuaries of Orissa) and also reported from Bangladesh and Myanmar. This fish (figure 1) has 66-78 anal soft rays, 21 or 22 keeled scutes at belly from isthmus to anus. Lower gill rakers with the serrae even or becoming clumped in some specimens. These fish derive their name from the long, filamentous extension of the pectoral fins that is found in most species (Setipinna: Latin, septem = seven + Latin, pinna, -ae = fin). The Adults of S. phasa feed mostly on mysids and small prawns where as juveniles mainly on copepods. They possibly breed throughout the year, with peaks in October and November.

Their large size makes them an attractive food fish. For the present study 10 fish samples of this species are collected each weighing about 100g and 20 ± 5cm in length.

Gudusia chapra (Hamilton 1822) is a shad of the Clupeidae family, under the order- Clupeiformes, under the class-Actinopterygii. This fish is available in rivers of India and Bangladesh affluent to the Bay of Bengal chiefly the Ganges and Brahmaputra systems and the Mahanadi River of Orissa. It is also reported from Nepal and Pakistan. This fish (figure 2) is found in middle and upper reaches of rivers and also occurs in ponds, beels, ditches and inundated fields. Its body is fairly deep; 26 to 29 scutes along belly. A single triangular pectoral axillary scale is seen. The tip of dorsal fin is depressed to behind. Hind margin of scales is smooth. Dark blotch is seen behind gill opening, often followed by a series of spots along flank. Gill rakers fine and numerous, increasing with size of fish (100 to 280 at 4 to 16 cm standard length). For the present study 10 fish samples of this species are collected each weighing about 75g and 15 ± 5cm in length.
Adult fishes were collected through local fisherman during monsoon from Bakkhali which is major site of marine capture fisheries of Hoogly estuarine system, South 24 parganas, West Bengal, India. They were kept in -4Cº and brought to the laboratory. Prior to extraction, the fishes were de-scaled, the fins, heads and other visceral part 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 (TL) were extracted following the method of Bligh and Dyer (26). A portion of the total lipid was subjected to Thin Layer Chromatography (TLC) using silica gel for lipid class separation according to Rouser et al. (27).

The NL components obtained from the samples were hydrocarbon (HC), wax ester (WE), steryl ester (SE), 1-O- alkyl-diacyl glycerol (ADAG), triacylglycerol (TG) and sterol (ST). Total phospholipids from the samples were classified into different classes viz., cardiolipin (CL), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI) and sphingomyelin (SPH) by one dimensional TLC (27) 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 glycerophosphatidylethanolamine. Fatty acid methyl esters (FAME) of TL, NL, GL and PL were prepared by transmethylation (18) and pure methyl esters thus obtained were redissolved in 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 with a BPX-70 megabore capillary column of 30mt length and 0.53mm i.d., equipped with Flame Ionization Detector (FID). Injection port and detector temperatures were 250°C and 300°C, respectively. Nitrogen was used as carrier gas and its flow rate was 6.2 ml/min. Oven temperature was programmed from 150°C - 240°C with a rate of 8°C/min. Initial and final time were kept 2 minutes and 20 minutes, respectively. Quantization was done by computer using specific Clarity Lite software. Identifications were made by comparison of retention times (RT) with those of standards. The protocol of the experiment is shown in figure 3.

![Flow diagram of the analysis of lipid and fatty acids of body flesh of fishes under study.](image)

3. Results
The results obtained from the various analyses are presented below following the major findings from each fish species and finally comparative findings among them.

3.1. *Setipinna phasa* (Hamilton 1822)
The total lipid (TL) of *S. phasa* is, 12.51 expressed as % w/w of wet tissue, presented in table 1. The TL fractions are tabulated in table 1 and figure 4A. Among the various
Table 1. Composition of various classes of total lipids, various fractions of neutral lipids and phospholipids obtained from body flesh of two edible marine fish: *Setipinna phasa* and *Gudusia chapra*.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Phasa (<em>Setipinna phasa</em>)</th>
<th>Khoira (<em>Gudusia chapra</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Lipids (TL)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.51</td>
<td>10.72</td>
</tr>
<tr>
<td>Neutral Lipids (NL)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94.7</td>
<td>94.31</td>
</tr>
<tr>
<td>Glycolipids (GL)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.72</td>
<td>2.24</td>
</tr>
<tr>
<td>Phospholipids (PL)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.49</td>
<td>3.43</td>
</tr>
<tr>
<td>Hydrocarbon (HC)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Wax ester (WE)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.15</td>
<td>0.36</td>
</tr>
<tr>
<td>Steryl ester (SE)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>1-O-Alkyl-2,3-diacylglycerol(ADAG)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.08</td>
<td>46.82</td>
</tr>
<tr>
<td>Triacylglycerol (TG)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.92</td>
<td>53.18</td>
</tr>
<tr>
<td>Total Sterol (ST)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.74</td>
<td>3.87</td>
</tr>
</tbody>
</table>

<sup>a</sup>Expressed as % w/w of wet tissue.
<sup>b</sup>Expressed as % w/w of total lipids.
<sup>c</sup>Expressed as % w/w of neutral lipids.
<sup>d</sup>Expressed as % w/w of phospholipid.

Fig. 4. Comparative distribution of different lipid fractions in *Setipinna phasa*.

fractions of total lipid NL is found to be predominant over the other fractions, such as PL and GL. The amounts of NL, GL and PL are 94.7, 0.72 and 4.49, respectively; expressed as % w/w of total lipid. Among the various fractions of NL, presented in table 1 and figure 4B, TG is found to be abundant and quantified as 57.92% w/w of NL, followed by ADAG measuring 42.08% w/w of NL. However the other NL fractions are present in minute quantity such as ST is 2.74%, WE is 0.15%, SE is 0.12% and HC is only 0.2% of w/w of NL. The comparative distribution of different PL...
fractions is presented in table 1 and figure 4C. 50% of the w/w of PL is expressed by PC in this fish followed by 23% CL, 13.8% PE, 7.32% PI, and 5.85% SPH.

Composition of twenty five fatty acids are quantified by open tube gas-liquid chromatography from the body flesh of *S. phasa* is presented in table 2. The amount of SFA is maximum in GL and PL, whereas MUFA is maximum in TL and NL. Dominant fatty acids in the lipid classes in this fish are palmitic acid (16:0), oleic acid (18:1), palmitoleic acid (16:1), stearic acid (18:0), eicosapentanoic acid (20:5ω3), and docosahexaenoic acid (22:6ω3). Among SFA, palmitic acid (16:0) is the predominant. Small amount of myristic acid (14:0) is also found to be present but the amount of behenic acid (22:0) and lignoceric acid (24:0) are very minute and even not detected in GL. The amount of odd chain saturates like pentadecanoic acid (15:0) and heptadecanoic acid or margaric acid (17:0) are quite less in TL and NL though more in GL such as 2.8% and 3.4%, respectively; expressed as w/w of each component in total fatty acids. Oleic acid (18:1) is the most abundant monounsaturated fatty acids, followed by palmitoleic acid (16:1) in all lipid fractions. 18:2 is the only detected DUFA in this fish, though present in minute quantity in all the lipid fractions.

**Table-2. Comparative distribution of Fatty acid in the Total Lipid (TL), Neutral lipid (NL), Glycolipid (GL) and Phospholipid (PL) of *Setipinna phasa* and *Gudusia chapra*.**

<table>
<thead>
<tr>
<th>Components</th>
<th>Total Lipid</th>
<th>Neutral lipid</th>
<th>Glycolipid</th>
<th>Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phasa</td>
<td>Khoira</td>
<td>Phasa</td>
<td>Khoira</td>
</tr>
<tr>
<td><strong>SATURATES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0 (Myristic acid)</td>
<td>2.5</td>
<td>11.0</td>
<td>4.4</td>
<td>12.3</td>
</tr>
<tr>
<td>15:0 (Pentadecanoic acid)</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>16:0 (Palmitic acid)</td>
<td>39.0</td>
<td>31.1</td>
<td>37.8</td>
<td>33.2</td>
</tr>
<tr>
<td>17:0 (Margaric acid)</td>
<td>0.3</td>
<td>1.5</td>
<td>0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>18:0 (Stearic acid)</td>
<td>6.1</td>
<td>4.6</td>
<td>6.5</td>
<td>6.3</td>
</tr>
<tr>
<td>22:0 (Behenic acid)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>24:0 (Lignoceric acid)</td>
<td>0.2</td>
<td>0.5</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Total</td>
<td>48.4</td>
<td>49.2</td>
<td>49.6</td>
<td>53.84</td>
</tr>
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<td><strong>MONOENES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:1 (Myristoleic acid)</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:1 (Pentadecenoic acid)</td>
<td></td>
<td>0.1</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>16:1 (Palmitoleic acid)</td>
<td>8.3</td>
<td>12.4</td>
<td>8.4</td>
<td>13.2</td>
</tr>
<tr>
<td>17:1 (Heptadecenoic acid)</td>
<td>0.3</td>
<td>3.4</td>
<td>0.3</td>
<td>3.0</td>
</tr>
<tr>
<td>18:1 (Oleic acid)</td>
<td>33.5</td>
<td>13.6</td>
<td>33.0</td>
<td>15.2</td>
</tr>
<tr>
<td>22:1 (Docosanoic acid)</td>
<td>0.03</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>24:1 (Nervonic acid)</td>
<td>0.2</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Total</td>
<td>42.53</td>
<td>30</td>
<td>42</td>
<td>32.2</td>
</tr>
<tr>
<td><strong>DIENES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:2 (Hexadecadienoic acid)</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2 (Linoleic acid)</td>
<td>0.4</td>
<td>0.9</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td>0.4</td>
<td>1.1</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>POLYENES</strong></td>
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<td></td>
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<tr>
<td>18:3ω3 (α-linolenic acid)</td>
<td>0.2</td>
<td>0.6</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>18:3ω6 (γ-linolenic acid)</td>
<td>0.02</td>
<td>0.2</td>
<td>0.02</td>
<td>0.2</td>
</tr>
<tr>
<td>20:3ω3 (Eicosatrienoic acid)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>20:4ω3 (Eicosatetraenoic acid)</td>
<td>1.3</td>
<td>2.1</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>20:4ω6 (Arachidonic acid)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>20:5ω3 (Eicosapentaenoic acid)</td>
<td>2.6</td>
<td>11.2</td>
<td>2.4</td>
<td>7.7</td>
</tr>
<tr>
<td>21:5ω3 (Heneicosapentaenoic acid)</td>
<td></td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:4ω6 (Arachidonic acid)</td>
<td>0.04</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>22:5ω3 (Docosapentaenoic acid)</td>
<td>0.8</td>
<td>1.6</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>22:5ω6 (Osbond acid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:6ω3 (Docosahexaenoic acid)</td>
<td>2.9</td>
<td>2.7</td>
<td>2.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Total</td>
<td>8.46</td>
<td>19.1</td>
<td>7.93</td>
<td>13</td>
</tr>
</tbody>
</table>

* First and second figures represent, carbon chain length: number of double bonds. The ω values represent the methyl end chain from the center of double bond furthest removed from the carboxyl end.

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Total seven different kinds of ω3 fatty acids such as 18:3ω3, 20:3ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:5ω3 and 22:6ω3 are detected in this fish (Table 2). The precursor for further desaturase and elongase system of fatty acid synthesis, ALA is detected in all fractions in adequate amounts. The only odd chain 21:5ω3 PUFA is detected from NL and GL fractions in minute quantity. Three ω6 fatty acids such as 18:3ω6, 20:4ω6 and 22:4ω6 are found in this fish (Table 2). The comparative distribution of ω3 and ω6 fatty acids in the different lipid fractions in this fish is presented in Table 7. The total amount of ω3 fatty acids in TL, NL, GL and PL are 8.0, 7.31, 6.6, and 23.1, respectively; showing clearly that the maximum amount of ω3 fatty acids are found in PL among the different TL fractions in this fish. The comparative distribution of ω3 and ω6 fatty acids, their ratio and AI, TI indices in the Total Lipid (TL), Neutral lipid (NL), Glycolipid (GL) and Phospholipid (PL) of Setipinna phasa and Gudusia chapra is presented in Table 3. DHA is the major PUFA in S. phasa. EPA is found to be the second largest PUFA in all lipid classes here. Occurrence of both EPA and DHA is also high in PL among the various lipid fractions. All together PUFA is most abundant in the PL than the other fractions in this fish. 5, 8, 11- eicosatrienoic acid (20:3ω9) is not detected in this fish.

3.2. Gudusia chapra (Hamilton 1822)

The TL of G. chapra is, 10.72 expressed as % w/w of wet tissue, presented in table 1. The TL fractions are tabulated in table 1 and figure 5A. Among the various fractions of total lipid NL is found to be predominant followed by PL and then GL. The amounts of NL, GL, and PL are 94.31, 2.24, and 3.43 respectively, expressed as % w/w of total lipid. Among the various fractions of NL (Table 1 and fig. 5B), TG is the most abundant and quantified as 53.18% w/w of NL, followed by ADAG measuring 46.82 % w/w of NL. However the other NL fractions are present in minute quantity such as ST is 3.87%, WE is 0.36%, SE is 0.18%, and HC is 0.4% of w/w of NL. The comparative distribution of different PL fractions is presented in table 1 and figure 5C. 48.31% of the w/w of PL is expressed by PC in this fish followed by 18.92% CL, 18.72% PE, 9.02% PI, and 5.01% SPH.
Twenty six fatty acids are quantified by open tube gas-liquid chromatography from the body flesh of *G. chapra* presented in table 2. The amount of SFA is maximum in GL followed by NL and PL, whereas MUFA is maximum in NL. According to percentage of occurrence the dominant fatty acids in the lipid classes in this fish are palmitic acid (16:0), oleic acid (18:1), stearic acid (18:0), eicosapentanoic acid (20:5ω3), palmitoleic acid (16:1), myristic acid (14:0), and docosahexaenoic acid (22:6ω3). Among saturated fatty acids (SFA), palmitic acid (16:0) is the predominant. Myristic acid (14:0) is also found to be present but the amount of behenic acid (22:0) and lignoceric acid (24:0) are very minute and all these three are not detected in PL. The amount of odd chain saturates like pentadecanoic acid (15:0) and heptadecanoic acid (17:0) are quite less in TL and NL but more in GL such as 5.6% and 6.0% expressed as w/w of each component in total fatty acids respectively. Oleic acid (18:1) is the most abundant monounsaturated fatty acids, followed by palmitoleic acid (16:1) in all lipid fractions. Among the DUFA in this fish, the amount of linoleic acid (18:2) is more than 16:2, though present in minute quantity in all the lipid fractions.

Total seven different kinds of ω3 fatty acids such as 18:3ω3, 20:3ω3, 20:4ω3, 20:5ω3, 21:5ω3, 22:5ω3, and 22:6ω3 are detected in this fish (Table 2). The precursor for further desaturase and elongase system of fatty acid synthesis, α-Linolenic acid (18:3ω3) is detected in all fractions in adequate amounts. The only odd chain 21:5ω3 PUFA is detected from GL fraction. Four ω6 fatty acids such as 18:3ω6, 20:4ω6, 22:4ω6, and 22:5ω6 are found in this fish (Table 4). The physiologically very important arachidonic acid (20:4ω6) is found to be the leading among the other ω6 counterparts in TL and NL but in GL and PL it is however not detected.

The comparative distribution of ω3 and ω6 fatty acids in the different lipid fractions in this fish is presented in table 3. The total amount of ω3 fatty acids in TL, NL, GL, and PL are 18.4, 12.3, 10.8, and 34.4 respectively, showing clearly that the maximum amount of ω3 fatty acids are found in PL among the different TL fractions in this fish. The total amount of ω6 fatty acids in TL, NL, GL, and PL are 0.7, 0.7, 0.7, and 0.3, respectively. The ω3/ω6 ratio of this fish is found to be high due to the presence of more amount of ω3 than ω6 counterpart. In the TL, NL, GL, and PL the ω3/ω6 ratio are 26.28, 17.57, 15.42, and 114.3, respectively. In *G. chapra*, EPA is found to be the major PUFA. DHA is found to be the second largest PUFA in all lipid classes except in PL where DHA is leading over EPA. Occurrence of both EPA and DHA is also high in PL among the various lipid fractions. Altogether PUFA is most abundant in the PL than the other fractions in this fish. 5, 8, 11-eicosatrienoic acid (20:3, ω9) is not detected in this fish.

### 3.3. Comparative account of lipids and fatty acids of above two fish species

Composition of various classes of lipids obtained from body flesh of *Setipinna phasa* and *Gudusia chapra* is presented in table 1. The TL of *S. phasa* is predominant than *G. chapra* and among the major fractions of TL, NL was found to be the most predominant followed by PL and GL in both cases. The GL fraction is found to be more in *G. chapra* than *S. phasa*. Among the major fractions of the NL, TG is found to be the chief followed by ADAG in both cases. The amount of TG is little more in *S. phasa* than *G. chapra* but rest other fractions are found to be more in *G. chapra* than *S. phasa*. Again, comparing the PL fractions in, PC is found to be leading followed by CL in both fishes. The CL, PC and SPH content in *S. phasa* is more than *G. chapra* and for PE and PI *vice versa*. Though there are little variations among the content of NL and PL fractions, it is evident from figure 6 that the distribution pattern of these fractions is same for both of these fishes.

Composition of twenty five fatty acids of *S. phasa* and twenty six fatty acids of *G. chapra* were presented in table 2. In both fishes the amount of SFA is maximum in GL.
MUFA is maximum in TL in *S. phasa* and NL in *G. chapra*. Among SFA, palmitic acid is the major one. Oleic acid is the most abundant MUFA. DHA in *S. phasa* and EPA in *G. chapra* are the major PUFA. In both of the fish, for all lipid classes, the amount of SFA was maximum than the rests and PUFA is predominant in PL (Fig.7). The amount of SFA ranges between 48.4% - 76.7% in *S. phasa* and 49.2% - 70.3% in *G. chapra* whereas MUFA ranges between 14.24% - 42.53% in *S. phasa* and 9.7% - 32.2% in *G. chapra*. Occurrence of EPA and DHA is also high in PL but for each of the lipid fractions, it is evident that EPA contents of *G. chapra* are predominant over that of *S. phasa*. Comparing DHA content, it can be said that in TL and NL, DHA contents of *S. phasa* are predominant but in GL and PL DHA of *G. chapra* is leading. Comparing ω3 fatty acids in table 3, it is evident that the ω3 content is high in PL followed by TL, NL and GL in both cases. In each of the lipid fraction ω3 content of *G. chapra* is more than *S. phasa* and the ω6 fatty acids in both of the fishes are present in small quantities in all lipid fractions.

**Fig. 6.** Comparative distribution of different NL and PL fractions in the muscle lipids of the fishes of present study

**Fig. 7.** Comparative distribution of different NL and PL fractions in the muscle lipids of the fishes of present study

**Fig. 7.** Comparative distribution of different fatty acid classes among different lipid classes of the fishes under study.
4. Discussion
The comparative discussion of lipid and fatty acids classes, their functions on fish physiology and importance in human nutrition are discussed accordingly. 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 (28). The amount of TL obtained from various fish was investigated and it was observed that the value ranges between 0.6-30% (29, 30, 31, and 32). Lambertsen (33) 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.

In the present study, it was found that S. phasa and G. chapra have TL content 12.51% and 10.72% respectively, reflecting that both of them have high fat content. The TL content in the muscle of four major fresh water food fish of India (Labeo rohita, L. calbasu, Catla catla, and Cirrhinus mirgala) was about to 1% and in Labeo bata it is 2.5% (25). In Hilsha (Tenualosa ilisha) which is one of the important commercial fish species in Hooghly estuarine system of West Bengal, values of TL content varies from 1.32% to 20.85% being a migratory fish (34). Purely marine fish species generally have higher level of lipids but in pomfret (Pampus argenteus) TL content in the muscle is 1.43% (35) and in Bombay duck (Harpadon nehereus) it is 2.1% (36) which are comparatively low though both of them are truly marine species.

Among the TL fractions, the NL contents of both the fish under study are predominant. The six components of NL of both the fish are showing the same pattern of distribution where TG content is found to be leading. In S. phasa it is 57.92% and in G. chapra little less (53.18%) amount of TG is present. TG is the storage lipids in almost all commercial fish species.

The other NL fraction total sterols (ST) are unsaponifiable, bicyclic hydrocarbons having a fused, tetracyclic, cyclopentanoperhydrophenanthrene ring system in their molecules which may be esterified with fatty acids to form steryl esters (SE) (18). In S. phasa ST and SE contents are 2.74% and 0.15% and in G. chapra they are 3.87% and 0.18% respectively. In fin fish 95% of the ST is cholesterol (6). Cholesterol is a major component of plasma membrane and metabolic precursor of steroid hormones and bile acids (37). Cholesterol is transported from the liver to extrahapatic tissues mainly in low density lipoprotein particles (LDL) of plasma and from extrahapatic tissues to the liver in high-density lipoprotein particles (HDL). In human, the average level of cholesterol is 175 mg/100ml of blood plasma. A high plasma LDL-cholesterol may lead to atherosclerosis by depositing cholesterol plaques on extrahapatic vascular walls (37).

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 can be converted to cholesteryl esters that are excreted mainly in the bile as in the non-aqueous central core of micelles formed by bile salts and PC. 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 (37). Therefore, fish with high cholesterol content should not be preferred as healthy.

Wax esters (WE) may also be a major component of some species' flesh and/or livers (38). In S. phasa and G. chapra 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 (39, 40). WEs 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, WEs aid in buoyancy and occur in higher amounts in roe lipids (41).

Glycolipids (GL) are fatty acid esters of sphingosine, carrying carbohydrate in addition, present in small quantity in both of the fish under study. In S. phasa only 0.72% and in G. chapra 2.24% GL were found. In Coilia reynaldi, Boleophthalmus boddartii and Dasyatis bleekerii GL contents were found in moderately higher percentage - 22.08% (42), 26.4% (43), and 22.9% (44) respectively. Phospholipids (PL) are fatty acid esters carrying a phosphate in addition. 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. 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 (45). There were altogether five components present in the PL fraction of which phosphatidylcholine (PC) is major in both cases. A typical fish polar lipid fraction contains about 60% PC, 20% PE, and several percent PI and SPH while the remainder is composed of other minor phospholipids (6). In fish the phospholipid PC: PE ratio is generally 2:3:1 (6). In S. phasa the PC: PE ratio is 3.62:1 which is slightly higher than the normal range. In G. chapra it is 2.58:1 belonging perfectly under the normal range.

The distribution of fatty acids in the various lipid fractions in both of the fish of under discussion is following almost same pattern with little variation as presented in table 2. Comparing the fatty acid classes in TL and NL of these two fishes, it is evident that in S. phasa, the MUFA content is greater but SFA, DUFA and PUFA contents are lesser than G. chapra but comparing GL, the SFA content of S. phasa is found to be predominant and MUFA, DUFA and PUFA contents are lesser than G. chapra. The SFA and MUFA contents of PL in S. phasa are greater than G. chapra whereas DUFA content of both fish is same and PUFA is predominant in G. chapra. In amadi (Coilia reynaldi), the amount of SFA is maximum in GL like the current fishes under study but PUFA is maximum in NL whereas PUFA is maximum in PL in the present investigation (42).

The common SFAs are straight-chain even-numbered acids containing 12-24 carbon atoms. Among the SFAs, lauric acid (12:0), myristic acid (14:0) and palmitic acid (16:0), are recognized as health risk factors as because they show a
tendency to increase the haematic cholesterol concentration. There is a very high correlation between the sum of these three acids and the thrombus formation (46). However, the lauric acid is not detected in any lipid classes of both the fishes. 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 both of the fish 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 S. phasa and G. chapra this acid is predominant and maximally found in GL. Stearic acid (18:0) is also relatively common and may on occasion be more abundant than palmitic acid, especially in complex lipids. In both cases this acid is moderately present and maximally found in GL.

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 (18). The presence of significant level of odd numbered fatty acids (15:0 and 17:0) in both of the fish is interesting. A possible explanation of the presence of these cyclopropane fatty acids (C17 and C19) are found in marine bacterium as well as in browser gastropods in freshwater (47). This C17 fatty acid might have taken by the fish through phytoplankton as because there is no known metabolic role for these fatty acids in vertebrates. Presence of significant amount of odd numbered fatty acids is also reported in Magil cephalus (48) and roe of Mugil parida (41). 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 (18).

Straight chain MUFAs of 15, 16, 17, 18, 22, 24 carbon containing one double bond have been characterized from both fish under study. Palmitoleic acid (16:1ω7) is a component of most animal fats and present in quite high concentration in these fishes. The family of polyunsaturated fatty acids, derived from palmitoleic acid, and odd-PUFA, have been found in some fish oils. Oleic acid or 18:1ω9 is found to be the predominant monoenoic acid in both cases. Oleic acid can also be the primary precursor of a family of PUFA. Biosynthetic relationships are, obvious as several components may arise by chain elongation or chain shortening of a common precursor. Diets reach in MUFAs resulted very efficient in reducing the coronary diseases risk. Indeed MUFAs have been recognized as beneficial as the PUFA’s ω3 class for human health because of their effect in lowering blood cholesterol.

In S. phasa only the dienoic linoleic acid or 18:2ω6 is present in minute quantity but in G. chapra, 16:2 and 18:2 both 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 synthesised by the animal yet is required for growth, reproduction and healthy development (49). There are many interacting external (temperature, salinity, food availability) and internal factors, including species, sex, physiological status (gonad maturity, condition, age) that determine and affect the PUFA content of aquatic organisms. 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 (50). Fish take up the ω3 PUFA from their food, as essential nutritional components, which they cannot synthesise 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) (51, 52).

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 FA between marine and freshwater fish. ALA is extremely important primary precursor of another important family of polyunsaturated fatty acids. In both of the fish under study, this acid is present. The metabolic conversion of ALA to its longer-chain products via the various desaturation/elongation reactions is observed in most freshwater fish and some marine species (53). As opposed to freshwater fish, marine species cannot convert C18 to C20:6ωPUFA; thus, AA is also an essential FA in marine fish. In consequence, it is interesting to mention that DHA can undergo to produce EPA via peroxisomal-mediated retroconversion (54).

The γ-Linolenic acid or 20:3ω6 (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 both of the fish under study, this acid is not found. 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. AA (cis-5, cis-8, cis-11, cis-14-eicosatetraenoic acid or 20:4ω6) is one of the principal precursors of a highly important series of hormone-like compounds known as prostaglandins (55). These compounds have profound pharmacological activity and are currently the subject of extensive 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 (18).

Fatty acid deficiency in fish species is indicated by the presence of 20:3ω9 acid (56). These fish were collected from estuary where natural foods were available in abundance. Thus, the absence of eicosatrienoic acid in these fish indicates that they are not suffering from fatty acid deficiency.

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 fish (54). The ω3 PUFA, also play an important role in
adaptation to changed environmental conditions and immunity to infections and parasitic diseases (54, 57). The ω3/ω6 ratio can be used to facilitate identification of high ω3 PUFA foodstuffs. In general, the ω3/ω6 ratio is higher for marine foodstuffs. 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 (53, 58, 59). 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) (57). 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 (60).

It has been suggested that a ratio of 1:1 to 4:1 would contribute to a healthy human diet (61) and WHO recommendation is the daily ratio of ω3/ω6 in total human diet should be more than 1.5 (62). However in both the fishes under study this ratio is far more than this range. The ω3/ω6 ratio in the TL, NL, GL and PL of S. phasa are 17.39, 11.79, 66.0, and 115.5, and of G. chapra are 26.28, 17.57, 15.42, and 114.3, respectively.

EPA and DHA, 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. (63) 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 (23, 64). The presence of low amounts of 20:4ω3 and much higher levels of 20:5ω3 and 22:6ω3 in all lipid fractions of both fish suggests that they can convert 20:4ω3 to 20:5ω3 to 22:6ω3.

The DHA plays an important role in reproduction 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 (57). 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 (57). There is a correlation between the fish DHA content and mobility of salt and freshwater fish (65, 57).

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.

Such conversion is very essential for maintenance of fish physiology (23, 64). 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) (66, 67). It is expected that in our body the alteration of metabolism of adhesion molecules prevents atherosclerosis and reduce the risks of CHD by removing these molecules from blood. This can be achieved by dietary intake of high PUFA containing fish.

The fat quality of these fish species were evaluated qualitatively by atherogenic index (AI) and thrombogenic index (TI) and quantitatively by serving frequency per week. AI is the ability to reduce the blood lipid content; and TI is the ability to reduce the platelet activity (46). In the present study it is evident from the data presented in table 3 and figure 8, that both the AI and the TI indices, for all lipid

**Fig. 8.** Qualitative assessment of muscle lipid by comparative AI and TI indices of the fishes under study
classes in both of the fishes, are present in fair amount. In S. phasa the TI indices ranges between 0.31 -0.88 and G. chakra this range is 0.23-1.0. This low values of TI indices for both fishes is suggesting a high anti-thrombogenic quality of fish meat in contrast to the TI values of lamb meat (1.4) and milk-based products (2.1) (68). 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 (69).

For patients with documented CHD, the American Heart Association guidelines advise 900-1000 mg/day of EPA/DHA combined (70, 71, and 72). They have indicated that for secondary preventions, this target would require one fatty fish meal per day or alternatively supplementation with EPA/DHA from fish oil sources. To meet the 900 mg/day for the patient with documented CHD, S. phasa requires 9 servings and G. chakra requires only 4 servings per week if 100g of fish meat is served in per serving.

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.

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**References**


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