

## Lipid classes, fatty acid and phospholipid composition of roe lipids from *Catla catla* and *Cirrhinus mrigala*

<sup>1</sup>Prabhakara Rao, P.G., <sup>1</sup>Balaswamy, K., <sup>1</sup>Narsing Rao, G., <sup>1</sup>Jyothirmayi, T., <sup>2</sup>Karuna, M.S.L. and <sup>2,\*</sup>Prasad, R.B.N.

<sup>1</sup>Central Food Technological Research Institute, Resource Centre, Habshiguda, Uppal Road, Hyderabad-500007, India

<sup>2</sup>Indian Institute of Chemical Technology, Centre for Lipid Research, Tarnaka, Hyderabad-500007, India

### Article history

Received: 18 May 2011  
 Received in revised form:  
 29 April 2012  
 Accepted: 30 April 2012

### Abstract

The lipid profiles and fatty acid composition of fish eggs (roes) from two common freshwater fish species viz., catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*) have been studied. The total lipid of the egg was extracted using chloroform/methanol (2:1, v/v) and was separated into neutral lipids, glycolipids and phospholipids using silica gel column chromatography. The fatty acid composition of the total lipid and the lipid classes was analysed by GC, GCMS and the phospholipids fraction was analysed by high performance liquid chromatography. The neutral and phospholipid fractions were 46.5 and 27.6% in catla, and 51.3 and 27.4% in mrigal. In total lipids, hexadecanoic acid was found to be the major fatty acid at 34.7 and 37.5% in catla and mrigal respectively. Octadecanoic acid was found to be 13.8 and 12.3% and octadecaenoic acid at 22.9 and 17.9% in catla and mrigal roe lipids. Eicosapentaenoic acid was found to be 2.0 and 0.5% while docosahexaenoic acid was to an extent of 6.9 and 8.9% in catla and mrigal respectively. The phospholipid fraction was rich in phosphatidylcholine and phosphatidylethanolamine as seen from HPLC analysis.

### Keywords

*Catla catla*  
*Cirrhinus mrigala*  
 fish roe lipids  
 lipid classes  
 fatty acid composition

© All Rights Reserved

### Introduction

Fish is well known for its nutritional value with essential fatty acids and its consumption is ever increasing. The health benefits of fish oils in prevention of cardiovascular diseases through modifications of risk factors like hyperlipidemia, hypertension, and secondary prevention following myocardial infarctions have been reviewed (Calder, 2004). They confirmed that n-3 PUFA present in fish oils decrease the mortality through antithrombic activity and possible atherosclerotic plaque prevention. Lipid composition of different parts of a mullet type fish (*Liza carinata*) such as roe, muscle and viscera were studied (Joe *et al.*, 1988). Roe lipids had high content of wax esters (63%) and low triglycerides (9.9%) in contrast to muscle which contained excess of triglycerides (66%) and traces of wax esters. Roe wax esters were significantly rich in monounsaturated fatty acids such as 16: 1, 18: 1 and polyunsaturated fatty acid, EPA (20: 5). Phospholipids were the major class of lipids in herring roe with palmitic acid (25.8%), oleic acid

(13.2%), docosahexaenoic acid (DHA) (21.6%) and eicosapentaenoic acid (EPA) (14.4%) and further studies concluded that a combination of lipid and protein helped to reduce the plasma lipids and fat pad at abdominal positions of rats (Moriya *et al.*, 2007). The lipid content of 6.6% (dry basis) was reported in southwest Atlantic Hake (*Merluccius hubbsi*) roe, of which 27.6% were waxes, 42% triacylglycerols, 14% phospholipids and 5.7% cholesterol. The PUFA were 45% of the total lipid, which makes this marine lipid an attractive substitute for PUFA enrichment in normal diets (Mendez *et al.*, 1992).

The lipid classes and fatty acid composition of eggs from the Atlantic halibut (*Hippoglossus hippoglossus*) were studied (Peterson *et al.*, 1986). They reported neutral lipids as a major class which constituted 30% of the total lipid. The phospholipid fraction contained 62% PC and 7 % PE with high concentration of n-3 PUFA such as EPA and DHA.

Various products based on caviar available in the market were reviewed (Bledsoe *et al.*, 2003). India is the third largest producer of fish in the world,

\*Corresponding author.  
 Email: [rbtnprasad@iict.res.in](mailto:rbtnprasad@iict.res.in)

and second in inland fish production (Anon, 2008). Freshwater fish species viz., catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*) are two major carps in India next to rohu (*Labeo rohita*). These carps formed the major species of the total inland fish production (953106 tonnes inland catch and 3479000 tonnes aquaculture) in India in 2008 (Anon, 2010). The present work was aimed to determine the lipid classes, fatty acid and phospholipid compositions of roes of catla and mrigal fishes.

## Materials and Methods

All chemicals, solvents used in this study were of analytical grade and were procured from Sd Fine Chemicals, Mumbai, India. Glass coated TLC plates (20 × 20 cm) were procured from Sigma-Aldrich, St. Louis, USA for the characterization of lipid classes. Standard fatty acid methyl esters (C 4 – C 24) and phospholipids components, PC, PE and PI were procured from Sigma-Aldrich, St. Louis, USA.

### *Sample collection, preparation and analysis*

Inland fish eggs (roes) from catla and mrigal were collected from 5 live fishes (each weighing about 1.5 kg) of each species freshly slaughtered in a local market during July, 2009. The roes of individual fishes were mixed for uniformity and kept under refrigeration (5-8°C) for less than 24 h before processing. The roes were cleaned to remove fins, scales and other blood vessels, and immediately ground with a blender into a homogenous mass. The homogenized material was analysed for moisture and fat content as per standard procedures (AOAC, 1990).

### *Extraction of lipid*

The homogenized material (100 g) was extracted with a mixture of chloroform-methanol at room temperature using a standard method (Folch *et al.*, 1957) to recover the total lipid maintaining a solid – solvent ratio of 1:4 in 3 successive extractions. The pooled solvent extracts were distilled in a rotary vacuum evaporator at  $\leq 50^\circ\text{C}$  to obtain the total lipid (24.4 g from catla and 21.9 g from mrigal roe). The lipid was stored in a refrigerator at 4-6°C for further analysis. The total lipid was analysed for free fatty acid content (FFA) as per standard methods (AOCS, 1991).

### *Separation of lipid classes by column chromatography*

The total lipids (TL) of catla and mrigal lipids (1 g) were separated into different lipid classes by silicic acid column chromatography. The column was successively eluted using chloroform, acetone

and methanol to recover neutral lipids, glycolipids and phospholipids respectively (Christie, 2003). The lipid fractions were qualitatively analyzed by TLC for identifying triglycerides, glycolipids and phospholipid components (Jacin and Mishkin, 1965).

Different combinations of solvent systems such as hexane : ethyl acetate (9:1, v/v) for neutral lipids, chloroform : methanol : acetic acid:water (170:24:25:4, v/v/v/v) for glycolipids and chloroform : methanol : water (65:25:4, v/v/v) for phospholipids were used. The eluted spots were identified by developing with spray reagents namely  $\alpha$  – naphthol for glycolipids and ammonium molybdate - perchloric acid for phospholipids (Mangold, 1961) Dragendorff reagent (Vashkovsky and Kostetsky, 1968) for phosphatidylcholine (PC) and lysophosphatidylcholine; ninhydrin (Mangold 1961); Vashkovsky and Kostetsky 1968) for phosphatidylethanolamine (PE). Fatty acid methyl esters (FAMES) of the total lipid and the individual lipid classes were prepared by transesterification using 2% sulphuric acid in methanol (Christie, 1993). The FAMES were extracted into ethyl acetate and thoroughly washed with water to make them free of acid and dried over anhydrous sodium sulphate. The dried esters were analysed in GC and GCMS. All the analyses were conducted in triplicate, standard deviation (SD) was computed in MS Excel 2003 and the data is presented as mean of triplicate values  $\pm$  SD.

### *Gas chromatography and gas chromatography - mass spectrometry*

The GC-FID analyses were performed with an Agilent (Agilent Technologies, Palo Alto, CA, USA) 6850 series gas chromatograph equipped with an FID detector using a DB-225 capillary column (30 m × 0.25 mm, 0.25  $\mu\text{m}$  of film thickness). The column temperature was initially maintained at 160°C for 2 min, increased to 220°C at 5°C/min and maintained for 10 min at 220°C. The carrier gas was nitrogen at a flow rate of 1.5 mL/min. The injector and detector temperatures were maintained at 230 and 250°C, respectively with a split ratio of 50:1. Identification of the fatty acids was based on comparison of GC retention times with those of standard reference fatty acid methyl esters performed under the same conditions.

The GCMS analyses were performed using an Agilent (Palo Alto, USA) 6890N gas chromatograph equipped with an HP-5 MS capillary column (30 m × 0.25 mm i.d.) connected to an Agilent 5973 mass spectrometer operating in the EI mode (70 eV; m/z

50 – 550; source temperature 230°C and a quadruple temperature 150°C). The column temperature was initially maintained at 200°C for 2 min, increased to 300°C at 4°C/min, and maintained for 20 min at 300°C. The carrier gas was helium at a flow rate of 1.0 mL/min. The inlet temperature was maintained at 300°C and split ratio of 50:1. Structural assignments were based on interpretation of mass spectrometric fragmentation and confirmed by comparison of retention times as well as fragmentation pattern of authentic compounds and the spectral data obtained from the Wiley and NIST libraries.

#### Quantification of phospholipids by high performance liquid chromatography

Individual phospholipids were quantified with normal phase Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump and an evaporative light scattering detector (ELSD 2000, Alltech, Deerfield, IL). The operating temperature of the ELSD was 50°C and nitrogen was used as the nebulizing gas at a flow rate of 1.5 L/min. HPLC separations were made on a LiChrosorb Si 60 (5 µm, 20 × 3 mm i.d., Merck, Darmstadt, Germany) at a solvent flow rate of 1 mL/min. A binary gradient system composed of eluent A [chloroform/methanol/28% ammonium hydroxide (80:19.5:0.5, v/v/v)] and eluent B [chloroform/methanol/28% ammonium hydroxide/water (60:34:0.5:5.5, v/v/v/v)] was used following the solvent elution profile: eluent A for 10 min; followed by linear increase in eluent B to 100% and held for 15 min. Identification of phospholipids was carried out by comparing them with the retention time of respective commercial standards. Calibration curves for each phospholipid was drawn by injecting different concentrations (5 – 40 µg/mL) and these were used to quantify the individual phospholipids following the method described earlier (Avalli and Contarini, 2005).

## Results and Discussion

#### Fat analysis

Fish roes from catla and mrigal analysed for moisture, fat and the physico-chemical characters of fat were presented in Table 1. The moisture in the samples was to the extent of 66.9 and 64.5% and the total lipid was 24.4 and 21.9% respectively in catla and mrigal roe samples on dry weight basis.

#### Chromatographic separation of lipid classes and fatty acid composition

The TLC of phospholipid fractions clearly

Table 1. Physico-chemical analysis of fresh fish roe and the total lipid<sup>a</sup>

Parameter (wt %)	<i>Catla catla</i>	<i>Cirrhinus mrigala</i>
Moisture	66.9 ± 0.66	64.5 ± 0.30
Total lipid content (dry matter basis)	24.4 ± 0.36	21.9 ± 0.66
FFA, as % oleic acid in total lipid	5.9 ± 0.36	3.8 ± 0.12

<sup>a</sup>Values are mean of triplicate analyses ± SD

Table 2. Composition of roe lipid classes<sup>a</sup>

Lipid Class	Composition (wt.%)	
	<i>Catla catla</i>	<i>Cirrhinus mrigala</i>
Neutral lipids	46.5 ± 0.46	51.3 ± 0.64
Glycolipids	25.9 ± 0.82	21.3 ± 0.30
Phospholipids	27.6 ± 0.70	27.4 ± 0.62

<sup>a</sup>Values are mean of triplicate analyses ± SD

indicated the presence of phosphatidylcholine by the characteristic orange spots by spraying with Dragendorff reagent after developing in chloroform: methanol: acetic acid (65:25:4, v/v/v) and pink spots on spraying phosphatidylethanolamine with ninhydrin. Phosphatidylinositol (PI) was present in minor amounts as seen from the TLC. The composition of lipid classes is presented in Table 2, which showed neutral lipids were higher (51.3%) in mrigal roe lipids and glycolipids are marginally higher in catla roe lipids (25.9%). However, the phospholipids content was almost identical in both catla (27.6%) and mrigal (27.4%) roe lipids.

The presence of higher percentage of PL fraction was reported in other 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). The dehydrated fish egg powders of rohu (*Labeo rohita*) and murrel (*Channa striatus*) studied by our group for lipid classes and fatty acid composition (Prabhakara Rao et al., 2010) showed 43.8, 72.9% neutral lipids; 12.7, 9.4% glycolipids and 43.5, 17.7% phospholipids. The fatty acid composition of total lipids, neutral lipids, glycolipids and phospholipids based on peak area percentages of GC FID data is shown in Table 3. The fatty acid profile of total lipids showed that the saturated fatty acids were found to an extent of 52.0 and 52.8% with major fatty acid being hexadecanoic acid to an extent of 34.7 and 37.5% in catla and mrigal eggs respectively. However, the saturated octadecanoic (13.8, 12.3%) and monounsaturated octadecenoic acid (22.9, 17.9%) were also present in considerable quantities. The octadecadienoic acid and octadecatrienoic acids were 3.3, 2.0% and 2.9, 6.0% in catla and mrigal lipids respectively. EPA (20:5) and DHA (22:6) were found to be 2.0, 6.9% and 0.5, 8.9% in catla and mrigal roe lipids respectively.

However, the presence of saturated fatty acids with palmitic acid in major quantities in the present

Table 3. Fatty acid composition (wt.%) of individual lipid fractions of fish roes<sup>a</sup>

Fatty acid	<i>Catla catla</i>				<i>Cirrhinus mrigala</i>			
	Total lipid	Neutral lipids	Glycolipids	Phospholipids	Total lipids	Neutral lipids	Glycolipids	Phospholipids
14:0	2.1 ± 0.45	1.80 ± 0.06	3.1 ± 0.02	2.2 ± 0.14	1.5 ± 0.13	1.5 ± 0.12	1.4 ± 0.09	0.7 ± 0.01
15:0	1.4 ± 0.05	1.1 ± 0.02	3.2 ± 0.07	1.3 ± 0.06	-	-	-	-
16:0	34.7 ± 3.50	31.6 ± 0.60	38.8 ± 0.79	36.2 ± 1.18	37.5 ± 1.14	38.0 ± 0.61	41.9 ± 1.20	42.4 ± 1.06
18:0	13.8 ± 0.10	12.9 ± 0.28	8.3 ± 0.13	15.0 ± 0.31	12.3 ± 0.33	13.5 ± 0.10	14.8 ± 0.87	17.3 ± 0.47
20:0	-	-	-	-	0.4 ± 0.04	0.6 ± 0.06	1.1 ± 0.08	0.2 ± 0.07
23:0	-	-	-	-	1.1 ± 0.06	-	1.0 ± 0.10	0.8 ± 0.03
Saturated	52.0	50.5	59.7	54.7	52.8	53.6	60.2	61.4
16:1	4.5 ± 0.09	2.9 ± 0.03	6.5 ± 0.15	4.5 ± 0.05	5.5 ± 0.14	6.3 ± 0.09	5.9 ± 0.10	5.3 ± 0.12
18:1	22.9 ± 0.26	25.6 ± 0.79	18.7 ± 0.36	19.4 ± 0.79	17.9 ± 0.46	19.0 ± 0.79	15.0 ± 0.46	16.9 ± 0.10
20:1	-	-	-	-	0.9 ± 0.02	1.5 ± 0.11	0.6 ± 0.05	0.4 ± 0.01
Monounsaturated	27.4	28.5	22.4	24.4	24.3	26.8	21.5	22.6
18:2	3.3 ± 0.04	3.8 ± 0.09	3.9 ± 0.02	3.2 ± 0.02	2.9 ± 0.04	3.2 ± 0.05	4.8 ± 0.21	1.9 ± 0.03
20:2	-	-	-	-	0.8 ± 0.03	1.2 ± 0.01	0.5 ± 0.02	0.6 ± 0.04
18:3	2.0 ± 0.08	1.8 ± 0.03	4.3 ± 0.04	1.7 ± 0.09	6.0 ± 0.28	9.4 ± 0.07	5.9 ± 0.10	3.7 ± 0.06
20:3	-	-	-	-	1.7 ± 0.02	0.5 ± 0.01	1.5 ± 0.01	1.1 ± 0.03
20:4	3.8 ± 0.04	3.2 ± 0.02	0.6 ± 0.02	3.2 ± 0.05	2.1 ± 0.28	1.7 ± 0.02	1.5 ± 0.02	2.1 ± 0.02
20:5 (EPA)	2.0 ± 0.10	2.1 ± 0.04	0.7 ± 0.02	2.0 ± 0.11	0.5 ± 0.02	-	0.5 ± 0.02	0.3 ± 0.02
22:5 (DPA)	2.6 ± 0.04	2.7 ± 0.08	1.9 ± 0.02	2.9 ± 0.03	-	-	-	-
22:6 (DHA)	6.9 ± 0.03	7.4 ± 0.19	6.5 ± 0.19	7.9 ± 0.05	8.9 ± 0.14	3.6 ± 0.09	3.6 ± 0.03	6.3 ± 0.05
Polyunsaturated	20.6	21.0	17.9	20.9	22.9	19.6	18.3	16.0

<sup>a</sup>Values are mean of triplicate analyses ± SD

Table 4. Composition of roe phospholipids<sup>a</sup>

Phospholipid	Composition (wt.% Phospholipids)	
	<i>Catla catla</i>	<i>Cirrhinus mrigala</i>
Phosphatidylcholine	55.7 ± 1.10	59.8 ± 0.89
Phosphatidylethanolamine	39.0 ± 0.78	37.2 ± 0.55
Phosphatidylinositol	5.3 ± 0.11	3.0 ± 0.09

<sup>a</sup>Values are mean of triplicate analyses ± SD

study was also observed in lipids of fresh and marine roes (Ghosh, 1997; Tocher and Sargent, 1984). Large quantities of palmitic acid were found in lipids of marine pollock fish by-products like head, skin frames and viscera (Tocher and Sargent, 1984). The presence of higher amounts of saturated fatty acids was also observed in sea urchin roes (Mol *et al.*, 2008).

The fatty acid composition of individual lipid classes (neutral, glyco- and phospholipids) showed considerable quantities of hexadecanoic acid (31.6, 38.8 and 36.2% in catla and 38.0, 41.9 and 42.4% in mrigala). Octadecanoic acid was 12.9, 8.3, 15.0% and 13.5, 14.8, 17.3% and octadecenoic acid of about 25.6, 18.7, 19.4% and 19.0, 15.0, 16.9% in catla and mrigala roe respectively. Octadecatrienoic acid was found to an extent of 1.8, 4.3, 1.7% and 9.4, 5.9, 3.7% in catla and mrigala respectively. EPA and DHA in the phospholipid fractions were 2.0, 7.9% in catla and 0.3 and 6.3% in mrigala respectively. Polyunsaturated fatty acids (EPA and DHA) were found to be more concentrated in the phospholipid fractions. EPA was found in smaller amounts while DHA was observed in significant amounts. The concentration of EPA and DHA in significant amounts in the PL fraction was also observed in other roe lipids studied earlier (Mukhopadhyay and Ghosh, 2003; Mukhopadhyay

and Ghosh, 2007; Mukhopadhyay *et al.*, 2004; Prabhakara Rao *et al.*, 2010).

The fish roes of rohu and murrel studied by our group (Prabhakara Rao *et al.*, 2010) showed the presence of eicosapentaenoic acid (20:5, EPA) to an extent of 1.1 and 1.0% and docosahexaenoic acid (22:6, DHA) to an extent of 14.1 and 6.8% respectively in the PL fraction. Tveiten *et al.* (2004) observed selective retention of DHA, 16:0 and arachidonic acid during the development of embryo in spotted wolf fish. Tocher and Sargent (1984) reported the presence of higher amounts of ω-3 fatty acid in herring, cod, haddock, whiting and sandy eel, saithe and capelin roes and also observed that PL fraction contained very high amounts of EPA and DHA.

#### Composition of phospholipids

The phospholipids components showed PC and PE in major quantities which accounted for 55.7 and 39% in catla and 59.8 and 37.2% in mrigala roes (Table 4). Higher amounts of PC with considerable amounts of PE and PI were also reported by Mukhopadhyay and Ghosh (2003), Mukhopadhyay *et al.* (2004) and Ghosh (1997). This further enhances the nutritive and medicinal importance of catla and mrigala roe lipids. The study reveals that roes of both *Catla catla* and *Cirrhinus mrigala* are good choices of food as they contain EPA and DHA in addition to saturated and monounsaturated fatty acids.

#### Acknowledgements

The authors thank the Department of

Biotechnology, Government of India, New Delhi for funding the project.

## References

- Anonymous. 2008. Agriculture Finance Corporation Ltd. Fishery sector in India. Mumbai, India. <http://www.afcindia.org.in>.
- Anonymous. 2010. The State of World Fisheries and Aquaculture 2010. Food and Agriculture Organisation, Rome, Italy.
- AOAC. 1990. Official methods of analysis of the Association of Official Analytical Chemists (15<sup>th</sup> edn.). AOAC International, Washington DC, U.S.A.
- AOCS. 1991. Official methods and recommended practices of the American Oil Chemists Society (4<sup>th</sup> ed.) American Oil Chemists Society. Washington DC, U.S.A.
- Avalli, A. and Contarini, G. 2005. Determination of phospholipids in dairy products by SPE/HPLC/ELSD. *Journal of Chromatography A* 1071: 185-190.
- Bechtel, P. J. Chantarachoti, A. C. M. and Sathivel, S. 2007. Characterisation of protein fractions from immature Alaska Walleye Pollock (*Theragra chalcogramma*) roe. *Journal of Food Science* 72: S338-343.
- Bledsoe, G. E., Bledsoe, C. D. and Rasco, B. 2003. Caviars and fish roe products. *Critical Reviews in Food Science and Nutrition* 43: 317-356.
- Calder, P. C. 2004. Long chain n-3 fatty acids and cardiovascular disease: further evidence and insights. *Nutrition Research* 24: 761-772.
- Christie, W. W. 1993. Preparation of ester derivatives of fatty acids for chromatographic analysis. in "Advances in Lipid Methodology - Two". (Christie, W.W. ed.) Oily Press: Dundee, Scotland, 69-111.
- Christie, W. W. 2003. Lipid Analysis. in "Isolation, separation, identification and structural analysis of lipids". (Christie W. W. ed.) The Oily Press: Bridgewater, England, 373-387.
- Folch, J., Lees, M. and Stanley, G. S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226: 497-509.
- Ghosh, M. 1997. Principal fatty acids of phospholipid classes of an Indian fresh water fish (*C. pabda*). *Journal of Food Lipids* 4: 189-197.
- Jacin, H. and Mishkin, A. R. 1965. Separation of carbohydrates on borate-impregnated silica gel G plates. *Journal of Chromatography* 18: 170-173.
- Joe, Y. G., Lee, K. H. and Cho, Y. J. 1988. Lipid composition of roe, muscle, and viscus of *Liza carinata*, a species of the mugilidae family. *Korean Journal of Food Science and Technology* 20: 674-682.
- Mangold, H. K. 1961. Thin-layer chromatography of lipids. *Journal of American Oil Chemists Society* 38: 708-727.
- Mendez, E., Fernandez, M., Pazo, G. and Grompone, M. A. 1992. Hake roe lipid: Composition and changes following cooking. *Food Chemistry* 45: 179-181.
- Mol, S., Baygar, T., Varlik, C. and Tosun, S. Y. 2008. Seasonal variations in yield, fatty acids and amino acids and proximate compositions of sea urchin (*Paracentrotus lividus*) roe. *Journal of Food and Drug Analysis* 16: 68-74.
- Moriya, H., Hosokawa, M. and Miyashita, K. 2007. Combination effect of herring roe lipids and proteins on plasma lipids and abdominal fat weight of mouse. *Journal of Food Science* 72: 231-234.
- Mukhopadhyay, T. and Ghosh, S. 2003. Lipid profile and fatty acid composition in eggs of common carp (*Cyprinus carpio*). *Journal of Oleo Science* 52: 439-442.
- Mukhopadhyay, T. and Ghosh, S. 2007. Lipid profile and fatty acid composition of two silurid fish eggs. *Journal of Oleo Science* 56: 399-403.
- Mukhopadhyay, T. Nandi, S. and Ghosh, S. 2004. Lipid profile and fatty acid composition in eggs of Indian feather back fish phouli (*Notopterus pallas*) in comparison with body-tissue lipid. *Journal of Oleo Science* 53: 323-328.
- Peterson, F. S., Peterson, F. I. B., Sargent, J. R. and Haug, T. 1986. Lipid class and fatty acid composition of eggs from the Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* 52: 207-211.
- Prabhakara Rao, P. G., Jyothirmayi, T., Karuna, M. S. L. and Prasad, R. B. N. 2010. Studies on lipid profiles and fatty acid composition of roe from Rohu (*Labeo rohita*) and Murrel (*Channa striatus*). *Journal of Oleo Science* 59: 515-519.
- Tocher, D. R. and Sargent, J. R. 1984. Analyses of lipids and fatty acids in ripe roes of some Northwest European marine fish. *Lipids* 19: 492-499.
- Tveiten, H., Jobling, M. and Andreassen, I. 2004. Influence of egg lipids and fatty acids on egg viability, and their utilization during embryonic development of spotted wolf-fish, *Anarhichas minor* Olafsen. *Aquaculture Research* 35(2): 152-161.
- Vashkovsky, V. E. and Kostetsky, G. Y. 1968. Modified spray for the detection of phospholipids on thin-layer chromatograms. *J. Lipid Res.* 9: 936.