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FATTY ACID FRACTIONS OF PHOSPHOLIPIDS IN AN INARTICULATE BRACHIOPOD FAUNA INHABITING IN WEST BENGAL- ODISHA COAST, INDIA.

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ABSTRACT

Different categories of good quality fatty acids under different classes of phospholipids are considered to be important determinants of ecosystem health and stability. They are also a valuable tool to measure inputs, cycling of materials in estuarine- mangrove food webs. *Lingula anatina*, a Precambrian brachiopod benthic macrofauna, inhabiting at the confluence of Subarnarekha estuary. They obtained their essential fatty acids (EFAs) of phospholipid groups, needed for their different physiological activities. These EFAs entered within their body through biotransformation and bioconversion processes from different food sources available in plenty at this intertidal basin. Different saturated and unsaturated fatty acids present within the body of studied species indicated that these can be considered as a good biomarker to understanding thophic interactions at a mangrove- estuarine ecosystem at the confluence of Subarnarekha estuary.

KEY WORDS: Brachiopod, Phospholipids, Biomarker, Subarnarekha Estuary.

INTRODUCTION

Lipids are major sources of metabolic energy and essential materials for the formation of cell and tissue membranes. They are very important in the physiology and reproductive processes of marine animals and reflect the special biochemical and ecological conditions of the marine environment.[1, 2, 3] The relative proportion and composition of fatty acids (FA) in marine organisms are characteristic for every species and genus. Several comprehensive reviews are available on marine FA, their occurrence, their roles and the methods used in their analysis.[4, 5, 6, 7, 8]

Marine phospholipids (PL) have been the focus of much attention recently. Many studies have shown that marine PL provides more advantages than marine triglycerides (TAG) available from fish oil. These advantages include: i) a higher content of physiologically important n-3 long chain polyunsaturated fatty acids (LC PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[9] ii) a better bioavailability of EPA and DHA[10] iii) a broader spectrum of health benefits including those from n-3 LC PUFA, their polar head groups and the combination of the two in the same molecule[11]iv) a better resistance towards oxidation due to the antioxidative properties of PL.[12, 13]

Phospholipids can be categorized into three major classes: glycerophospholipids, ether glycerolipids and sphingophospholipids. Among them, glycerophospholipid is the most widespread class and serves as ecologically important biomarker to understanding marine-estuarine food web interactions among different floral and faunal community. Therefore, the emphasis is given on phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and sphingomyelin (SPM) to document various types of saturated and unsaturated fatty acids of above phospsolipid groups of *Lingula anatina*.

Bilayer structures of phospholipids are main constituents of biological membranes, which serve as biological boundaries, responsible for metabolic regulation.[14] Marine phospholipids also contain high amounts of pharmaceutically significant polyunsaturated –ω3 fatty acids, mainly eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). The interest in marine phospholipids as carriers of –ω3 fatty acids increases as –ω3 fatty acids from phospholipids were observed to be more easily accessible for catabolic processes than –ω3 fatty acids from triglycerides.[15]

In marine ecosystem, lipids provide the densest form of energy which is transferred from algae to vertebrates via zooplankton. [16] Alongside they contain essential fatty acids and sterols which are considered to be important drivers of ecosystem health and stability. The importance of different fatty acids and phospholipids with different head groups in regulation of cellular processes together with the fact that fluidity may be controlled by just a few compounds suggests that molecular species analysis would also help describe mechanism behind the ecological effects of essential fatty acids.[16]

Studies on fatty acids of phospholipids have been carried out previously by various authors in various animals like Freites *et al*.(2002) , Caers *et al*. (1999) and Soudant *et al*. (1999) in mussels; Soudant *et al*. (1999) and Abad *et al*.(1995) in Oysters; Kraffe *et al*. (2004) and Fernandez-Reiriz *et al*. (1999) in clams and Pazos *et al*. (1997) in molluscs; Hayashi and Kishimura (2002) in squids; Suprayudi *et al*.(2004) , Hamasaki *et al*. (2002) and Lahdes *et al*.(2000) in crustaceans and Litchfield and Morales (1976) and Barnathan and Kornprobst (1998) in sponges, Parrish(2009) in echinoderms and Vertebrates. Phospholipids, the most important lipid classes have been studied in bivalves also.[31] But no studies have been carried out in intertidal brachiopod benthic animal such as *Lingula anatina* inhabiting in three contrasting mudflats at Talsari at the confluence of Subarnarekha estuary. The present study aims focusing the qualitative composition of saturated and unsaturated fatty acids of four classes of phospholipid's groups in *Lingula anatina*.

MATERIOLS AND METHODS

Individuals of *Lingula anatina* were collected randomly from three contrasting study sites (namely SI, SII, and SIII) from the confluence of Subarnarekha estuary Bay of Bengal at Talsri (Longitude 87º5′ E to 88º5′ E and Latitude 20º30′ N to 22º2′ N) near New Digha, West Bengal, India. Muscles of *Lingula anatina* were separated by dissection in the laboratory and immediately frozen and stored at -20°C until analyzed. The study was undertaken during premonsoon period, 2011.

Total lipids were extracted from each sample following Bligh and Dryer (1959) method. Identification and confirmation of fatty acids were done by following Ackman (1989). The same methodology as published by Das et al, 2014 and Samanta *et al*., 2014 for total lipid extraction and identification and confirmation of fatty acids were also followed during present research work.

RESULTS

Biochemical analysis of muscles of samples of *Lingula anatina* have revealed that among 3 major classes of lipids (viz.-neutral lipid-NL, glycolipid –GL and phospholipids -PL), phospholipids were recorded highest amount during present study as documented in Table 2. Further analysis of PL exhibited 4 different classes of phospholipids i.e. PE, PC, SPM and PS which have been found to be present within the muscles of *L.anatina* as represented in Table 3. Among these PC have been recorded in highest amount (32.84%, Table 3).

Various classes of fatty acids (as found during present research), their designation, names, recent uses as biomarkers and their documentation by several authors, under 4 major groups of phospholipids are represented in the Table 4 and 5. From this it is found that *Lingula anatina* contained 7 different types of saturated fatty acids (SAFA), 7 mono unsaturated fatty acids (MUFA) and 14 different types of polyunsaturated fatty acids (PUFA). Out of 14 different types of PUFA 21:5ω3 registered highest amount (11.9%; under PS group) followed by 20:ω3 (6.8%; under PC group) and 20:4ω6 (5.9%; under PE group and 2.4% under SPM group).

The present paper has documented that the studied animal contained good amount of PUFA (79.09%) out of which 47.70% belongs to -ω3 categories and 34.02% belongs to –ω6 categories. From Table 5 it is also inferred that the *L. anatina* possessed very high amount of SAFA.

Among different categories of SAFA 16:0 was recorded in highest amount (PC- 40.8%, SPM- 38%, PE- 35.5% and PS- 14.6%) in all types of phospholipid groups. Out of 7 various classes of MUFA 18;1ω9 recorded highest amount (PC- 8.2%; PE- 4.1% and SPM- 3.9%) followed by 22:1 (1.6%) as expressed in Table 4. During present work it was found that among 14 different classes of PUFA 20:4ω6 was documented its highest value in PE group (5.9%) and SPM group (2.4%) where as 20:5ω3 showed its highest concentration (6.8%) in PC group and 21:5ω3 has showed its maximum peak (11.9%) in PS group as showed in Table 5.

Results of the analysis of different categories of phospholipids as recorded and presented in Table 5 has indicated that major fatty acid components in muscles of studied macrofauna were 16:0(max- 40.8%, PC), 18:0 (max- 37.1%, SPM), 18:1ω9 (max- 8.2%, PC), 18:2ω6 (max-4.2, PC), 18:3ω3(max-2.3%, PC), 20:4ω6 (max- 5.9%, PE) and 20:5ω3(6.8% PC). The amount of 22:6ω3 was quite low during present study as presented in Table 4 and 5. Fatty acid designations, names and some recent uses as biomarkers has been presented in the Table 6.

Table 1- Percentage of total lipid obtained from muscle of *Lingula anatina.*

Sample	Amount taken	Total lipid obtained
Muscles	4.32 gm	0.12 gm (2.78%)

Table 2- Percentage of various classes of lipid obtained from total lipid of muscle of *Lingula anatina.*

Samples	Percentage of lipid (W/W)
Phospholipids (PL)	55.13
Neutral Lipids (NL)	32.57
Glycolopids (GL)	12.30

Table 3- Phospholipid composition of phospholipid fraction obtained from the total lipid of *Lingula anatina.*

Samples	Lipid obtained (mg)	Percentage of lipid (w/w0)
Phosphatidylethanolamine (PE)	10.6	12.09
Phosphstidylcholine (PC)	28.8	32.84
Sphingomyelin (SPM)	9.9	11.29
Phosphatidylserine (PS)	2.8	3.19

Table 4: Total amount of different fatty acids obtained from different classes of phospholipids.

Name of fatty acids	Percentage of fatty acids (w/w)
PUFA	79.02
-ω3	47.70
-ω6	34.02

Table 5: Fatty acids compositions of various classes of phospholipids obtained from muscle sample of *Lingula anatina* (% w/w of each component in total fatty acids).

Components [a]	PE	PC	SPH	PS
14:0	4.6	2.4	1.4	
15:0	1.2	0.7	0.6	
16:0	35.5	40.8	38.0	14.6
17:0	6.1	2.3	4.3	10.2
18:0	23.1	10.1	37.1	13.6
22:0	0.5	0.8	0.4	3.5
24:0	3.0	0.9	0.01	5.7
Total SAFA	74.00	58.00	81.81	47.60
14:1				
15:1	0.3	0.1	0.1	
16:1	2.2	12.8	1.4	
17:1	0.6	0.1	0.4	
18:1ω9	4.1	8.2	3.9	
22:1	0.2	0.1	0.7	1.6
24:1		0.1	1.6	0.4

Total MUFA	7.4	21.4	8.1	2.0
16:2	0.4	0.2	0.1	
17:2	0.3	0.3		
18:2ω6	4.2	2.6	1.8	9.3
18:3ω6	0.2	0.1	0.1	
18:3ω3	0.1	2.3	0.1	8.7
20:3ω3	1.5	0.2	0.6	
20:4ω6	5.9	3.2	2.4	5.5
20:4ω3	0.2	1.1	0.5	1.0
22:4ω6	0.5	0.5	0.3	0.6
20:5ω3	1.9	6.8	1.8	1.1
21:5ω3	0.5	0.1	0.2	11.9
22:5ω6	0.3	0.02	0.1	5.7
22:5ω3	0.3	0.7	0.4	1.8
22:6ω3	0.6	1.9	0.8	0.2
Total PUFA	16.9	20.02	9.2	32.9
Total –ω3	5.1	13.1	4.4	21.1
Total –ω6	11.1	6.42	4.7	11.8
PUFA/SAFA	0.22	0.34	0.11	0.69

[a] *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.*

Table 6- Fatty acid designations, names and some recent uses as biomarkers.

Components [a]	**Fatty acid Name**	**Biomarker for**	**Recent referances**
14:0	Myristic	Protobacteria, Diatoms, Prymnesiophytes	[35]
15:0	Pentadecanoic	Phytoplancton	[36]
16:0	Palmitic	Mangrove leaves	[34]
17:0	Margaric	Bacteria	[8]
18:0	Stearic	Mangrove leaves	[34]
22:0	Behenic	Terrestyrial Plants	[37]
24:0	Lignoceric	Mangrove and Terrestrial Plants	[38]
14:1	Tetradedecenoic	Proteobacteria	[35]
15:1	Pentadecenoic	Bacteria.	[39]
16:1	Palmitoleic	Planktons, Mangroves	[16, 34]
17:1	Heptadecenoic	Bacteria	[36]
18:1ω9	Oleic	Crustacea, Deep Sea fish, Macroalgae, Mangrove, Carnivory	[40]
24:1	Tetracosanoic	Zooplzncton	[38]
16:2	Hexadecadienoic	Planktons, Mangrove leaves	[34]
17:2	Heptadecadienoic	Planktons	[34]
18:2ω6	Linoleic	Mangrove, Sea grass, Macroalgae, Vascular plants,	[40]
18:3ω6	γ linolenic	Macro algae	[40]

18:3ω3	α linolenic	Mangrove, Sea grass, Vascular plant	[40]
20:3ω3	Eicosatrienoic		
20:4ω6	Arachidonic	Protozoa, Microeukaryotes, Red algae, Kelp	[40]
20:4ω3	Eicosatetraenoic	Fungi, Protozoa, Algae	[40]
22:4ω6	Adrenic	Phytoplanktons (Euglenophyceae)	[41]
20:5ω3	Eicosapentaenoic	Diatom, Brown and Red Macro algae	[40]
21:5ω3	Heneicosapentaenoic	Planktons	[34]
22:5ω6	Osbond, ω6DPA	Phytoplanktons(Ocromonadeles, Cryptophyceae)	[41]
22:5ω3	Clupanodonic, DPA	Diatoms	[42]
22:6ω3	Docosahexaenoic	Zooplancton	[40]

[a] *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.*

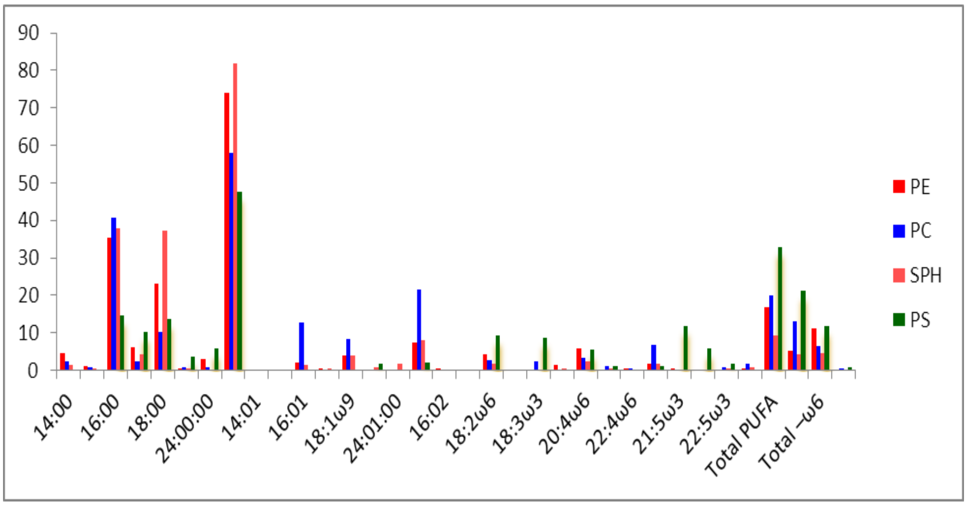

Figure 1: Fatty acids compositions of various classes of phospholipids obtained from muscle sample of *Lingula anatina* (% w/w of each component in total fatty acids).

DISCUSSION

Aquatic ecosystems occupy the largest part of the biosphere, and lipids in those systems provide the densest form of energy yielding at least two third more energy per gram than proteins and carbohydrates. They are highly reduced compounds and are thus important fuels for oxidation. [16] Lipids are also a solvent and absorption carrier for fat soluble vitamins, carotenoids and organic contaminants. Thus the study of lipid flow among trophic levels is important for models of both population dynamics and of bioacuumulation of hydrophobic chemicals.[16] Fatty acids are important not only for their impact on animal growth but also many other facets of functions like reproduction, immunity, ion balance regulation and even buoyancy regulation[43] and buoyancy control.[44] There are two related families of fatty acids (FA) consisting of ω3 and ω6 PUFAs. FAs in each of the families are interconvertible ususlly through alternate use of desaturases and elongases. The extent to which a given species at a given life stage can convert one ω3 FAs to another or one ω6 FAs to another determines the degree of essentiality of the FA for that species at that life stage.[16] In marine fauna essential FAs like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have very important functions at various trophic levels. [45] For example EPA is thought to be a key factor in buoyancy control. Probably for this, the studied animal exhibited successful infaunal succession as observed by Samanta *et al.,* 2014. EPA and DHA are has been found as stress resistant.[46] Therefore these animals after being kept in an unearthed condition in a bucket in the laboratory showed that they were capable to live successfully for 8-10days. Arachidonic acid (AA, 20:4ω6) is one example whose essentiality has often been overlooked.[47] AA enters several metabolic pathways in invertebrates.[48] Presence of AA in body part of studied species further indicated that detritus serves as one part of food of *L. anatina* and this is also supported by Kelly and Scheibling,2012, M¨uller-Navarra, 2004 and Samanta *et al*. 2014. In addition to AA there is another ω6 long chain PUFA i.e. docosapentaenoic acid (DPA, 22:5ω6) which may be essential in marine fauna It may play an important structural role in membranes and/or may be a precursor of bioactive docosanoids via enzymatic oxygenation.[16]

Few studies have shown a clear and direct relationship between unsaturated fatty acids and membrane fluidity in marine organisms.[49] While phospholipid molecular species containing DHA are believed to be important in controlling membrane fluidity as reported earlier by (Farkas *et al*., 2000). Hall *et al*., 2002 reported that there is very strong relationship between membrane fluidity and EPA. Low values of the PUFA/SAFA ratio (Tables-5 as determined in the present research investigation are because of the presence of high levels of palmitic acid (16:0), suggesting a contribution of vegetal detritus in the diet of *L. anatina* which corroborates the findings of Reemtsma *et al.*, 1990 and Samanta *et al*.,2014. Samanta *et al*., 2014 reported on the occurrence of 16:0, 18:0, 16:2, 17:2, 21:5ω3 in the planktons, mangrove leaves which were consumed by studied animals and hence they were also detected within the muscles of *L. anatina* which further strengthen the present findings.

Biological markers are receiving widespread attention in aquatic ecological environments.[53] Among several biochemical markers lipids and fatty acids can be used as signatures of individual organisms or of groupings of organisms or of certain environmental processes. From this point of view it can be concluded that presence of different classes of fatty acids (ALA, EPA, DHA, AA, etc) within the body of studied species derived through biotransformation and bioconversion processes from different sources like mangrove leaves, planktons and suspended materials on which they feed. Presence of 15:0 in the muscles of *L. anatina* indicated that they are plankton feeders and they collect it from suspended particulate matters avilabe in sufficient amount in the studied tidal basin. Presence of 18:1ω9 further indicated that it was entered within the body of studied fauna from degraded mangrove leaves through biotrnsformation process. From table 5 it is cleared that *Lingula anatina* captured foods from various sources like phytoplanktons (15:0), zooplanktons (24:1 and 22:6ω3), diatoms (20:5ω3, 14:0), brown and red macro algae (20:5ω3, 18:3ω6), sea grass (18:2ω6, 18:3ω3) and mangrove plants (18:3ω3, 20:5ω3, 18:2ω6, 18:1ω9, 24:0) etc which are available in plenty within and surrounding their habitats at Talsari near Subarnarekha estuary. The heterogeneous nature of phospholipids means that much information can be generated by determining individual classes of fatty acids of different groups of phospholipids. Individual class of fatty acids such as EPA or DHA of certain phospholipids' category viz. PE, PC, SPM or PS may be used to indicate the presence of certain types of organisms as well as their physiological state and activity. Lipid classes can also be used to indicate sources of organic matter[54] including dissolved organic matter and hydrophobic contaminants.[55] Lipid class information is particularly valuable when used in conjugation with determination of individual fatty acids.[56] Information provided by fatty acid biomarkers may be used to delineate carbon cycling and transfer of materials through food webs.[16]

CONCLUSIONS

Present investigation has revealed that muscles of the studied animal, *Lingula anatina* stored major amount of 4 categories of phospholipids. Fractionation analysis of these phospholipids exhibited that studied species contained various classes of good quality MUFA and PUFA and they obtained these through biotransformation and food chain food web interactions.

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REFERENCES

1. Sargent J R. Origins and functions of egg lipids: nutritional implications. In: Bromage N R, Roberts R J (eds.), Broodstock Management and Egg and Larval Quality, Oxford; Blackwell Science., 1995; 353.
2. Sargent J R, Bell M V, Bell J G, Henderson R J, Tocher D R. Origins and functions of n-3 polyunsaturated fatty acids in marine organisms. In: Cevc G, Paltauf F (eds.), Phospholipids: Characterization, Metabolism and Novel Biological Applications. AOCS, Illinois; Champaign, 1995; 248.
3. Pazos A J, Roman G, Acosta C P, Sanchez J L, Abad M. Comp Biochem Physiol, 1997; 117B: 393.
4. Ackman R G. Fatty acids. In: Ackman R G (eds). Marine biogenic lipids, Fats and Oils. CRC press Inc. Boca Raton., 1989; 2: 145-178.
5. Rezanka T. Progress in Lipid Research, 1989; 28: 147.
6. Christie W W. Preparation of derivates of fatty acids. In: Barnes J and associates (3rd eds.). Lipid analysis: isolation, separation and structural analysis of lipids., 2003; 205- 225.
7. Hagen W and Auel H. Seasonal adaptations and the role of lipids in oceanic zooplankton. Zoology, 2001; 104: 313–326.
8. Dalsgaard J, St John M, Kattner G, Müller-Navarra D, Hagen W. Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol, 2003; 46: 225-340.
9. Peng J L, Larondelle Y, Pham D, Ackman R G, Rollin X. Polyunsaturated fatty acid profiles of whole body phospholipids and triacylglycerols in anadromous and landlocked Atlantic salmon (Salmo salar L.) fry. Comparative Biochemistry and Physiology, 2003; Part B, 134: 335–348.
10. Wijendran V, Huang M C, Diau G Y, Boehm ., Nathanielsz P W, Brenna J T. Efficacy of dietary arachidonic acid provided as triglyceride or phospholipid as substrates for brain arachidonic acid accretion in baboon neonates. Pediatric Research, 2002; 51: 265–272.
11. Ierna M., Kerr A, Scales H, Berge K, Griinari M. Supplementation of diet with krill oil protects against experimental rheumatoid arthritis. BMC Musculoskeletal Disorders, 2010; 11.2010.

12. Cho S Y, Joo D S, Choi H G, Nara E, Miyashita K. Oxidative stability of lipids from squid tissues. Fisheries Science, 2001; 67: 738-743..
13. Moriya H, Kuniminato,T, Hosokawa M, Fukunaga K, Nishiyama T, Miyashita K. Oxidative stability of salmon and herring roe lipids and their dietary effect on plasma cholesterol levels of rats. Fisheries Science, 2007; 73: 668–674.
14. Berdanier C D. Fatty acids and membrane function. In: Chow C K, Dekker M (eds). Fatty acids in foods and their health implications, New York, US., 2000; 569-580.
15. Løvaas E. Marine phospholipids (MPL): resources, applications and markets. In: Luten J B, Jacobsen C, Bekaert K, Sæbø A, Oehlenschlager J(eds). Seafood research from fish to dish: quality, safety and processing of wild and farmed fish,The Netherlands: Wageningen Academic Publishers., 2006.
16. Parrish C C. Lipids in marine ecosystem. Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, NI, Canada AIC5S7. ISRN Ocenography., 2013; 16.
17. Freites L, Fernandez-Reiriz M J, Labarta U. Fatty acid profiles of *Mytilus galloprovincialis* (Lmk) mussel of subtidal and rocky shore origin. Comp. Biochem. Physiol, 2002; 123: 453–461.
18. Caers M, Coutteau P, Sorgeloos P. Impact of starvation and of feeding algal and artificial diets on the lipid content and composition of juvenile oysters (*Crassostrea gigas*) and clams (*Tapes phillippinarum*). Mar. Biol, 2000; 136: 891–899.
19. Soudant P, Ryckeghem K V, Marty Y, Moal J, Samain J F , Sorgeloos P , 1999. Comparison of the lipid class and fatty acid composition between a reproductive cycle in nature and a standard hatchery conditioning of the Pacific Oyster *Crassostrea gigas*. Comp. Biochem. Physiol, 1999; 123B: 209–222.
20. Abad M, Ruiz C, Martínez D, Mosquera G, Sánchez J L. Seasonal variations of lipid classes and fatty acids in flat oyster, *Ostrea edulis*, from San Cibran (Galicia Spain). Comp. Biochem. Physiol, 1995; 110C: 109–118.
21. Kraffe F, Soudant P, Marty Y. Fatty acids of serine, ethanolamine, and choline plasmalogens in some marine bivalves. Lipids, 2004; 39: 59-66.
22. Fernández-Reiriz M J, Labarta U, Albentosa M, Pérez-Camacho A. Lipid profile and growth of the clam spat, *Ruditapes decussatus* (L.), fed with microalgal diets and cornstarch. Comp. Biochem. Physiol, 1999; 124B: 309–318.

23. Pazos J A, Román G, Acosta C P, Sánchez J L, Abad M. Lipid classes and fatty acid composition in the female gonad of *Pecten maximus* in relation to reproductive cycle and environmental variables. Comp. Biochem. Physiol, 1997; 117B: 393–402.
24. Hayashi K, Kishimura H. Amount and composition of diacyl glyceryl ethers in various tissue lipids of the deep-sea squid *Berryteuthis magister*. J. Oleo. Sci, 2002; 51: 523–530.
25. Suprayudi M A, Takeuchi T, Hamasaki K. Essential fatty acids 839 for larval mud crab *Scylla serrata* :implications of lack of the 840 ability to bioconvert C18 unsaturated fatty acids to highly 841 unsaturated fatty acids. Aquaculture, 2004; 231: 403–416.
26. Hamasaki K, Suprayudi M A, Takeuchi T. Effect of dietary n-3HUFA on larval morphogenesis and metamorphosis to megalops in the seed production of mud crab, *Scylla serrata* (Brachyura: Portunidae). Susian Zoshoku, 2002; 50: 333–340.
27. Lahdes E. Balogh G. Fodor E. Farkas T. Adaptation of composition and biophysical properties of phospholipids to temperature by the Crustacean, *Gammarus* spp. Lipids, 2002; 35: 1093-1098.
28. Litchfield C, Morales R W. Are Demospongiae membranes unique among living organisms? In: Harrison F W, Cowden R K (eds), New York; Academic Press: 1976, pp. 183-200.
29. Barnathan G, Bourgougnon N, Kornprobst J M. Phytochemistry, 1998; 47: 761.
30. Parrish C C, Deibel D, Thompson R J. Effect of sinking spring phytoplankton blooms on lipid content and composition in suprabenthic and benthic invertebrates in a cold ocean coastal environment. Marine Ecology Progress Series, 2009; 391: 33–51.
31. Ekin İ. and Başhan M. Fatty acid composition of selected tissues of *Unio elongatulus* (Bourguignat, 1860) (Mollusca: Bivalvia) collected from Tigris River, Turkey. Turkish J Fish Aquat Sci, 2010; 10: 445.
32. Bligh E G, Dyer W J. A rapid method of total lipid extraction and purification. Can.J.Biochem.Physiol, 1959; 37: 911-917.
33. Das T K, Samanta S, Chakraborty S K. Pharmacologically active fatty acids of fiddler crab *Uca acuta acuta* (Simpson). International journal of lifesciences and pharma research, 2014; Vol-4(2): 21-27.
34. Samanta S, Das T K, Choudhury A, Chakraborty S. K. Lipid and fatty acid fractions in *Lingula anatina* (Brachiopoda): an intertidal benthic fauna in the West Bengal-Orissa coast, India. Journal of coastal life medicine, 2014; 2(5): 382-388.

35. Berge J P, Barnathan G. Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. Advances in Biochemical Engineering/Biotechnology, 2005; 96: 49–125.
36. Dalsgaard J, St John M, Jattner G, Müller-Navarra D, Hagen W. Fatty acid trophic markers in the pelagicmarine food environment. Adv Mar Biol, 2003; 46: 226–340.
37. Connelly T L, Deibel D, Parrish C C. Trophic interactions in the benthic boundary layer of the Beaufort Sea Shelf, Arctic Ocean: combining bulk stable isotope and fatty acid signatures. Progress in Oceanography, 2014; 120: 79- 92.
38. Rossi S, Isla E, Fietz S, Mart´ınez-Garcia A, Sa˜n´e E, Teixid`o N. Temporal variation of seston biomarkers within the Humboldt Current Systemoff northern Chile (21∘S): first simultaneous records on fatty acids, *n*-alkanes and glycerol-dialkylglycerol- tetraethers (GDGT). Advances in Oceanography and Limnology, 2012; 3: 17–40.
39. Joseph M M, Renjith K R, Ratheesh Kumar C S, Chandramohanakumar N. Assessment of organic matter sources in the tropical mangrove ecosystems of Cochin, Southwest India. Environmental Forensics, 2012; 13: 262–271.
40. George E M, Parrish C C. Invertebrate uptake of lipids in the vicinity ofAtlantic salmon (*Salmo salar*) aquaculture sites in British Columbia. Aquaculture Research, In press.
41. Kelly J R, Scheibling R E. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series, 2012; 446: 1–22.
42. Taipale S, Strandberg U, Peltomaa E, Galloway A W E, Ojala A, Brett M T. Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. Aquatic Microbial Ecology, 2013; 71: 165- 178.
43. Volkman J K, Jeffrey S W, Nichols P D, Rogers G I, Garland C D. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol, 1989; 128: 219–240.
44. Glencross B D. Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture, 2009; 1: 71–124.
45. Pond D W, Tarling G A. Phase transitions of wax esters adjust buoyancy in diapausing *Calanoides acutus*. Limnology and Oceanography, 2011; 56(4): 1310–1318.
46. Muller-Navarra D C, Brett M T, Park S et al. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature, 2004; 427(6969): 69–72.
47. Montero D, Kalinowski T, Obach A et al.. Vegetable lipid sources for gilthead seabream (*Sparus aurata*): effects on fish health. Aquaculture, 2003; 225(1–4): 353–370.

48. Bell J G, Mc Evoy L A, A. Estevez A, Shields R J, Sargent J R. Optimising lipid nutrition in first-feeding flatfish larvae. Aquaculture, 2003; 227(1–4): 211–220.
49. Ciapa B, Allemand D, Renzis G D. Effect of arachidonic acid on Na+/H+ exchange and neutral amino acid transport in sea urchin eggs. Experimental Cell Research, 1995; 218(1): 248–254.
50. Arts M T, Kohler C C. Health and condition in fish: the influence of lipids on membrane competency and immune response. In: Arts M T, BrettM T, Kainz M J (eds). Lipids in Aquatic Ecosystems, Dordrecht,The Netherlands; Springer: 2009, chapter 10, pp. 237–255.
51. Farkas T, Kitajka K, Fodor E et al.. Docosahexaenoic acidcontaining phospholipid molecular species in brains of vertebrates. Proceedings of the National Academy of Sciences of the United States of America, 2000; 97(12):6362–6366.
52. Hall J M, Parrish C C, Thompson R J. Eicosapentaenoic acid regulates scallop (*Placopecten magellanicus*) membrane fluidity in response to cold. Biological Bulletin, 2002; 202(3): 201–203.
53. Reemtsma T, Haake B, Ittekkot V, Nair RR, Brockmann U H. Downward flux of particulate fatty acids in the Central Arabian Sea. Mar. Chem, 1990; 29: 183-202.
54. Bianchi T S, Canuel E A. Chemical Biomarkers in Aquatic Ecosystems, Princeton University Press, 2011.
55. Marty J C, Goutx M, Guigue C, Leblond N, Raimbault P. Short-term changes in particulate fluxes measured by drifting sediment traps during end summer oligotrophic regime in the NW Mediterranean Sea. Biogeosciences, 2009; 6: 887–899.
56. Bourguet N, Goutx M, Ghiglione J F et al.. Lipid biomarkers and bacterial lipase activities as indicators of organic matter and bacterial dynamics in contrasted regimes at the DYFAMEDsite, NW Mediterranean. Deep-Sea Research II, 2009; 56(18): 1454–1469.
57. Galois R, Richard P, Fricourt B. Seasonal variations in suspended particulate matter in the Marennes-Oleron Bay, France, using lipids as biomarkers. Estuarine, Coastal and Shelf Science, 1996; 43(3): 335–357.