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Bio- conversion of the α-linolenic acid (18:3ω3) to long chain -ω3 PUFA's by detritivore fiddler crab, *Uca acuta acuta* in the mangrove-estuarine system at Midnapore (East), West Bengal, India

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Abstract

The present paper has attempted to highlight the transmission of bio-chemicals especially the omega-3 fatty acids from selected coastal mangrove leaf detritus in the process of foraging activities of *Uca acuta acuta*, a very common bioenergetically significant mangrove estuarine intertidal fiddler crab (Brachyura : Ocypodidae) inhabiting in the coastal belt of Midnapore (East),West Bengal, India and their bio-conversion in vivo. Leaf detritus has been found to contain appreciable amount of ω3 fatty acids, particularly, the α- linolenic acid (18:3ω3) which is consumed and bio transformed by this studied fiddler crab. On analyzing, the fatty acids from the flesh of the crab species, exhibited considerably higher levels of both 20:5ω.3 (EPA, Ecosapentaenoic acid) and 20:6 ω.3 (DHA, Docosahexaenoic acid) in various tissues of the animal. This intertidal benthic fauna has shown the ability of chain elongation which appeared to be a prerequisite for the de-saturation of the α-linolenic acid (18:3ω3) which in turn produces the essential long chain PUFA's (Poly Unsaturated Fatty Acids), EPA and DHA.

Key words : Fiddler Crab, Mangrove, Lipid Bio - Conversion, PUFA's (EPA and DHA).

INTRODUCTION

Biochemical studies on three selected species of mangrove and associated plants, viz. *Avicennia marina* (Family-Avicenniaceae), *Acanthus ilicifolius* (Family-Acanthaceae) and *Suaeda maritima* (Family- Chenopodiaceae), mangrove litters derived detritus and detritivore fiddler crab *Uca acuta acuta* (Stimpson,1858) belonging to the family Ocypodidae of the class Malacostraca is necessary for evaluating their nutritional values as well as the possibility of bio-conversion of specific lipid components.

Edible wild plants provide α-linolenic acid (ALA) and higher amounts of vitamin E and vitamin C than cultivated plants. In addition to the antioxidant vitamins, edible wild plants are rich in phenols and other compounds that increase their antioxidant capacity[1]. α-linolenic acid (ALA) is the parent compound of the omega-3 fatty acid family. It must be obtained from our diets because our bodies do not make it[2]. It is incorporated into cell membranes, promotes the health of blood vessels [3]. ALA is converted to the long-chain omega-3 fatty acids, especially ecosapentaenoic acid (EPA) and docosapentaenoic acid (DHA)[4].

The primary food source for aquatic and intertidal mangrove dwelling faunal components is derived from the detritus of the mangrove leaves. The breakdown of mangrove leaves is brought about by the activities of microorganisms, such as, fungi, bacteria and protozoa. Such litter decomposition process by the activities of benthic fauna (macro and microfauna), microarthropodes and microbes (fungi, bacteria etc) have been highlighted by several researchers[5,6,7,8,9]. ,

Among the intertidal macrobenthic fauna, brachyuran crabs constitute a dominant faunal component of ecological and economic significance[10, 11, 12]. 26 species of brachyuran crabs belonging to 15 genera and 5 families have been recorded from the deltaic Sundarbans mangrove estuarine complex[13], of which the species under present study appeared to be very significant from the point of view of its density and biomass[14].

In the present study, efforts have been made to determine various lipid components of these selected plant species, detritus and the body parts of the fiddler crab, *Uca acuta acuta*, a very common and abundant macro-benthic intertidal faunal component in the coastal mangrove estuarine complex of West Bengal[9] from the selected study area of Midnapore (East) costal belt, West Bengal, in between the Latitude 21°47′(N) and Longitudes 87°45′(E) with special reference to bio-transformation of specific lipid components (α-linolenic acid) from these selected mangrove plants to specific detritivore animal (Fiddler crab: *Uca acuta acuta*) and their bio-conversion have been undertaken.

MATERIALS AND METHODS

The Mangrove plant leaves, detritus and detritivore animal *Uca acuta acuta* were collected from the coastal belt of Purusattampur (Dadanpatrabar), Midnapur(East), West Bengal (Latitude 21°47′N and longitudes 87°45′E). The plant leaves, detritus and the i) Body muscles, ii) Largest Chela Flesh and iii) Hepatopancreas were dissected out of the body of a common fiddler crab species, *Uca acuta acuta* which were immediately frozen and stored at -20°c until analyzed.

Extraction of Lipids

The total lipids were extracted from the samples followed by the method of [15]using methanol chloroform (2:1,v/v), methanol-chloroform water (2:1:0.8, v/v/v), and then again with the first solvent system. Samples were grounded with the solvent, in a high speed homogenizer, filtered and residue was extracted with, the next solvent system. The process was repeated. Finally, the three extracts were pooled, diluted with water and layer was allowed to separate in a separatory funnel. The chloroform layer

at the bottom was withdrawn and dired over anhydrous sodium sulphate in a freezer. The chloroform solution of lipid was evaporated under vacuum, redissolved in distilled n-hexane and kept at -20°C for future use. BHT (Butylated Hydroxy Toluene) was added at a level of 100mg/L to the solvent as antioxidant. After dilution of the pooled extracts, a heavy white precipitate appeared at the junction of the two layers which were kept for further analysis.

Preparation of Methyl Esters of Fatty Acids

Total lipids were transformed into methyl esters by trans-methylation. The samples were dissolved in anhydrous methanol containing concentrated Sulfuric acid (1.0%, v/v) and the mixture were refluxed[16]for two hours. Methanol was evaporated to a small volume and cooled. Distilled water was added to the cooled mixture and the methy1 esters of Fatty acids were extracted 3 times with aliquots of diethy1 ether. The ethereal extracts were pooled and dried over anhydrous sodium sulfate, filtered, vacuum dried, dissolved in n-hexane and kept in a freezer for further use.

Purification of Fatty Acids Methyl Esters by Thin Layer Chromatography (TLC)

Fatty acid methyl esters were purified[17] by TLC using a solvent system of n-hexane-diethyl ether (90:10,v/v). A standard methyl ester was also run on the same plate in a separate lane. The location of methyl ester bands corresponding to the standard were marked after placing the TLC plate in an iodine vapour chamber and then scrapped off the methyl ester band from the plate. Methy1 esters were recovered by extracting the recovered bands in a mini glass column with chloroform, the later was evaporated and the Methy1 esters were kept in n-hexane in a freezer, till analyzed by GLC.

Gas Liquid Chromatography (GLC)

GLC of fatty acid Methy1 esters were done on a Chemito 1000 instrument, equipped with Flame Ionization Detector (FID).

Table 1: Fatty acid compositions of total lipid (TL) obtained from L1 (*Avicennia marina*), L2 (*Acanthus ilicifolius*), L3 (*Suaeda maritima*), and detritus samples as determined by GLC of methyl esters (% w/w of each component in total fatty acids).

Components[a]	L1	L2	L3	Detritus
14:0	0.5	1.1	0.5	6.7
14:1				2.6
15:0	1.4	1.0	1.8	2.6
15:1		0.3	0.5	0.5
16:0	35.2	22.1	29.7	38.3
16:1	1.5	1.8	1.3	10.9
16:2		0.4		1.0
17:0	0.9	0.8	0.4	0.4
17:1	0.4	0.3		0.2
18:0	3.5	4.9	5.1	4.5
18:1 ω9	14.4	13.1	2.3	13.1
18:2 ω6	10.3	21.9	17.3	9.0
18:3 ω6	1.1	0.1	0.2	1.6
18:3 ω3	30.1	31.0	39.6	2.7
20:3 ω3				0.3
20:4 ω6				0.2
22:0	0.1	0.1		0.3
22:1				0.1
20:4 ω3	0.1	0.3	0.4	1.2
22:4 ω6	0.04	0.1	0.04	
20:5 ω3				1.1
22:5 ω6				0.03
24:0		0.2	0.2	0.7
22:5 ω3				0.02
22:6 ω3				0.2

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

Quantitation was done by computer using specific clarity lite software.

Analysis of Fatty Acid Methy1 Ester (FAME)

GLC of FAME was done on a BPX-70 megabore capillary coloumn of 30 mt length and 0.53 mm i.d. obtained from SGE, Australia. Oven temperature was programmed from 150°C - 240°C with a rate of 8°C/min. Initial and final times were kept isotheremal for 1 minute and 20 minutes, respectively. Injection port and detector temperatures were 250°C and 300°C, respectively. Nitrogen gas was used as carrier gas, its flow being 6.32 ml/min.

Identification of Fatty acids was done by comparing their retention times with those of standards, chromatographed under identical operational conditional of GLC. Confirmation of fatty acids were also done by using the FAME of Cod liver oil fatty acids, as suggested[18-19]

RESULTS

The present investigation has revealed that these three mangrove plant species contain a considerable amount of pharmacologically active α-linolenic acid, the primary precursor molecule for the synthesis of ω3 family of fatty acids in animal tissues[4, 20]. About 25 fatty acids have been found to occur in these

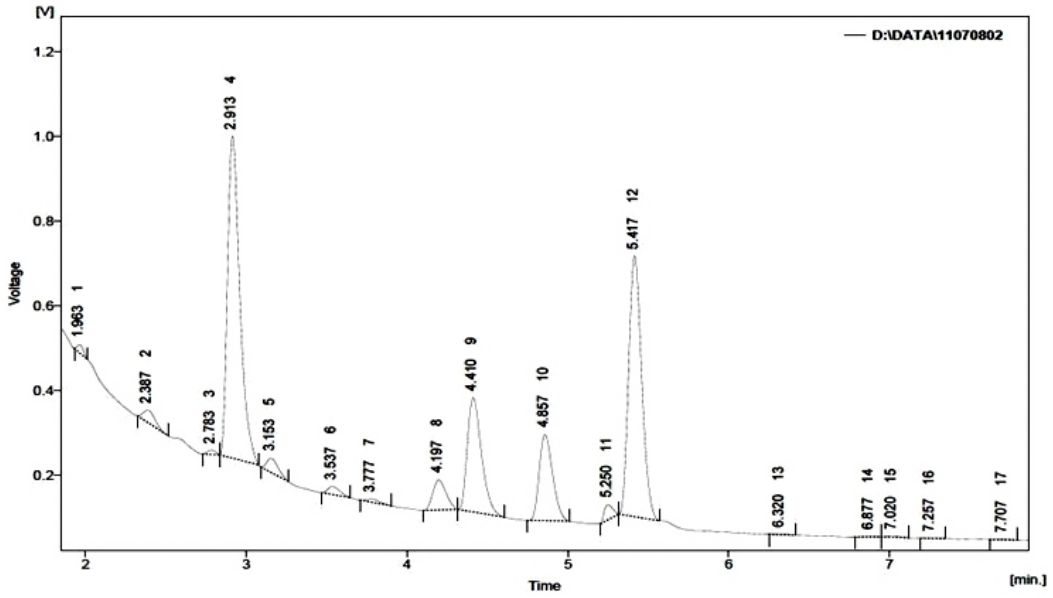


Figure 1: GLC tracing of the fatty acid methyl esters (FAMEs) of the leaf, *Avicennia marina* (L1). GLC column used was BPX-70 (Polar column) Megabore column (30 meters length x 0.530 mm dia).

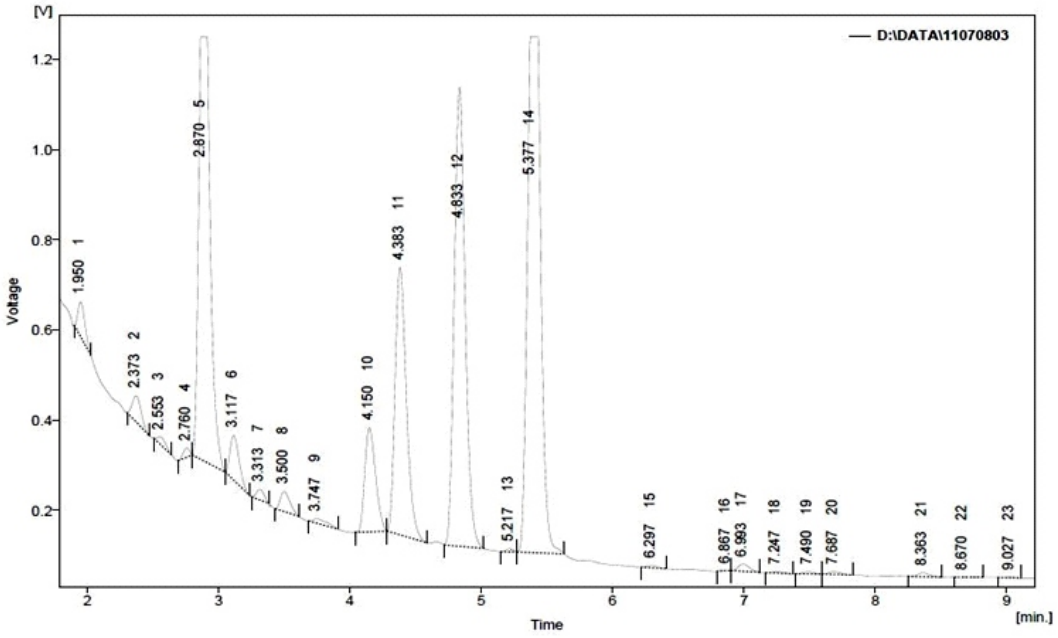


Figure 2: GLC tracing of the fatty acid methyl esters (FAMEs) of the leaf, *Acanthus ilicifolius* (L2). GLC column used was BPX-70 (Polar column) Megabore column (30 meters length x 0.530 mm dia).

leaf extracts and detritus (Table-1& Fig-1,2, 3 and 4).

Among the unsaturated fatty acids, the major component was α-linolenicacid (39.6%) in the L3(*Suaeda maritima*) , 31.0% in L2(*Acanthus ilicifolius*) ,30.1% in L1(*Avicennia marina*) and it was only 2.7% in the detritus .

Biochemical analysis on different parts of fiddler crab have revealed that the body flesh of *Uca acuta acuta* contains a considerable amount of both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The occurrence of considerably higher levels of EPA and DHA in the body flesh is common in detritivorous benthic animals of Sundarban estuarine complex [21].About 28 fatty acids have been found to occur in the body flesh, big chela flesh and hepatopancreas of *Uca acuta acuta* (Table-2 & Fig-5, 6 & 7).

Among the unsaturated fatty acids, the major component was eicosapentaenoic acid (22.3%) in the body flesh, 14.3% in big

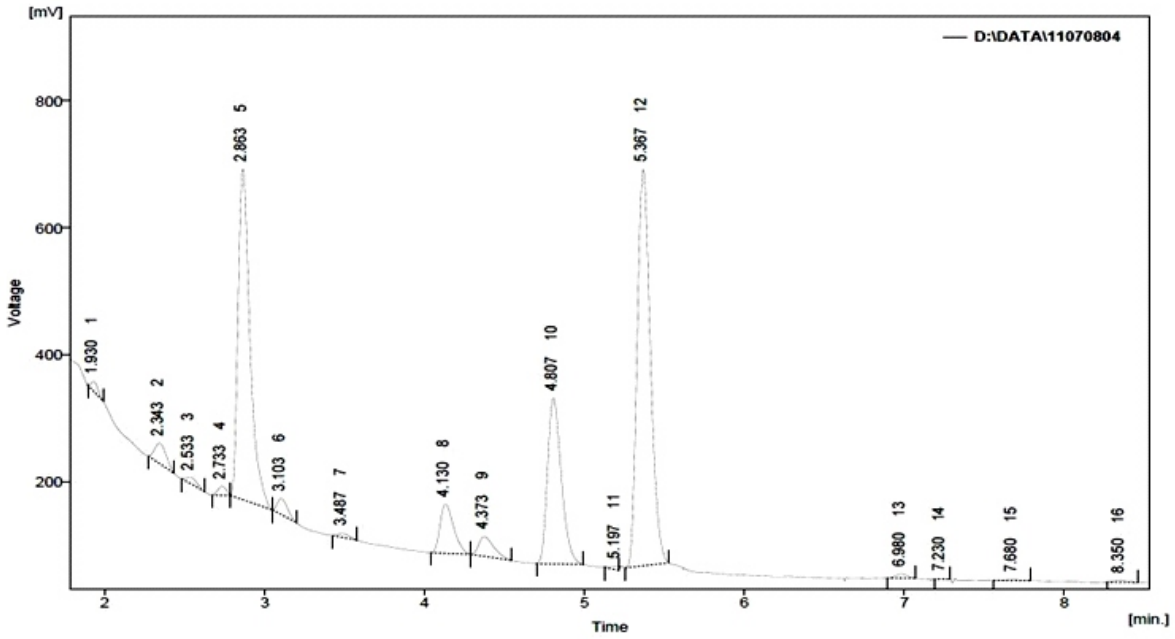


Figure 3: GLC tracing of the fatty acid methyl esters (FAMEs) of the leaf *Suaeda maritama* (L3).GLC column used was BPX-70 (Polar column)Megabore column (30 meters length x 0.530 mm dia)

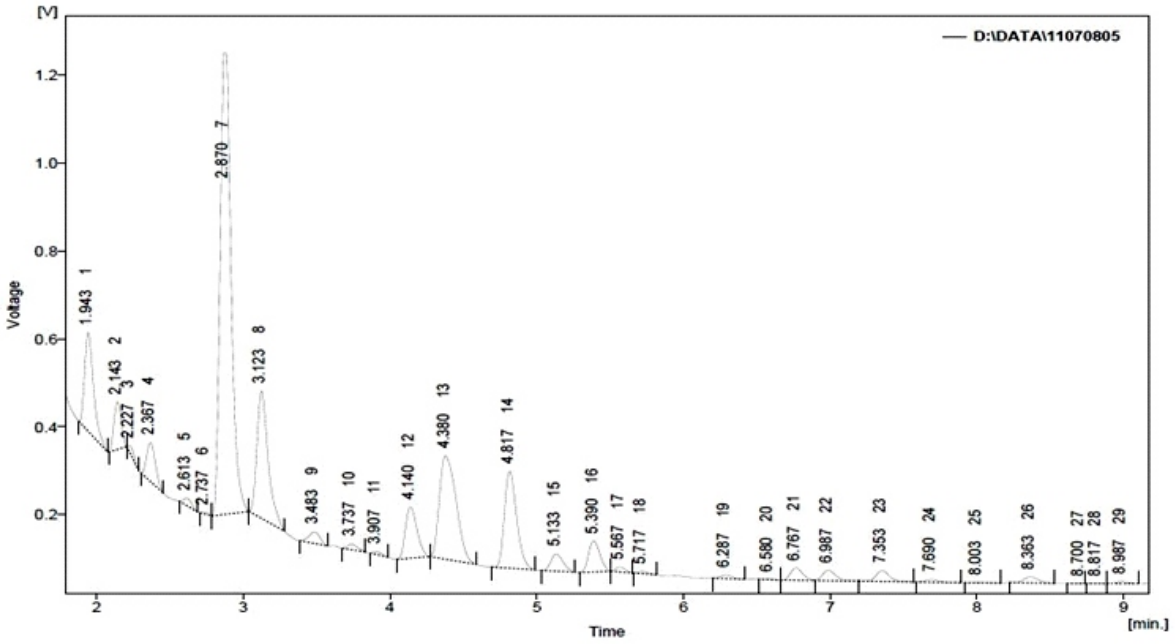


Figure 4: GLC tracing of the fatty acid methyl esters (FAMEs) of the detritus of the leaves. GLC column used was BPX-70 (Polar column)Megabore column (30 meters length x 0.530 mm dia).

Table 2: Faty acid compositions of Total Lipids (TL) from *Uca acuta acuta* (Fiddler Crab) as determined by GLC of methyl esters (% w/w of each component in total fatty acids).

[a] First and second figures represent, carbon chain length: number of double bonds.

Components[a]	Body Flesh	Big Chela Flesh	Hepatopancreas
13:0		1.0	0.2
14:0	2.2	4.3	6.9
14:1	2.3		0.1
15:0	0.7	4.5	0.4
15:1	21.6		0.3
16:0	9.9	18.7	13.6
16:1	0.2	8.0	10.3
16:2	2.5		2.5
17:0	0.6	1.3	3.0
17:1	9.4	2.6	3.9
18:0	6.3	12.4	10.7
18:1ω9	2.9	7.2	9.6
18:2ω6	0.5	4.7	4.4
18:3ω6	0.3	0.4	1.2
18:3ω3	0.2	0.3	1.0
20:3ω3	0.1	1.5	1.0
20:4ω6	0.4		0.7
22:0	0.1	0.1	0.3
22:1	6.2	1.0	0.3
20:4ω3	0.1	8.4	9.8
22:4ω 6	22.3	0.1	0.2
22:5ω 3	0.1	14.3	13.3
21:5ω 3			0.2
22:5ω 6			0.007
24:0	0.7	0.5	1.0
24:1	0.4	1.0	0.7
22:5ω 3	0.1	0.2	0.2
22.6ω	9.3	7.0	4.8

The -ω values represent the methyl end chain from the center of double bond furthest removed from the carboxyl end.

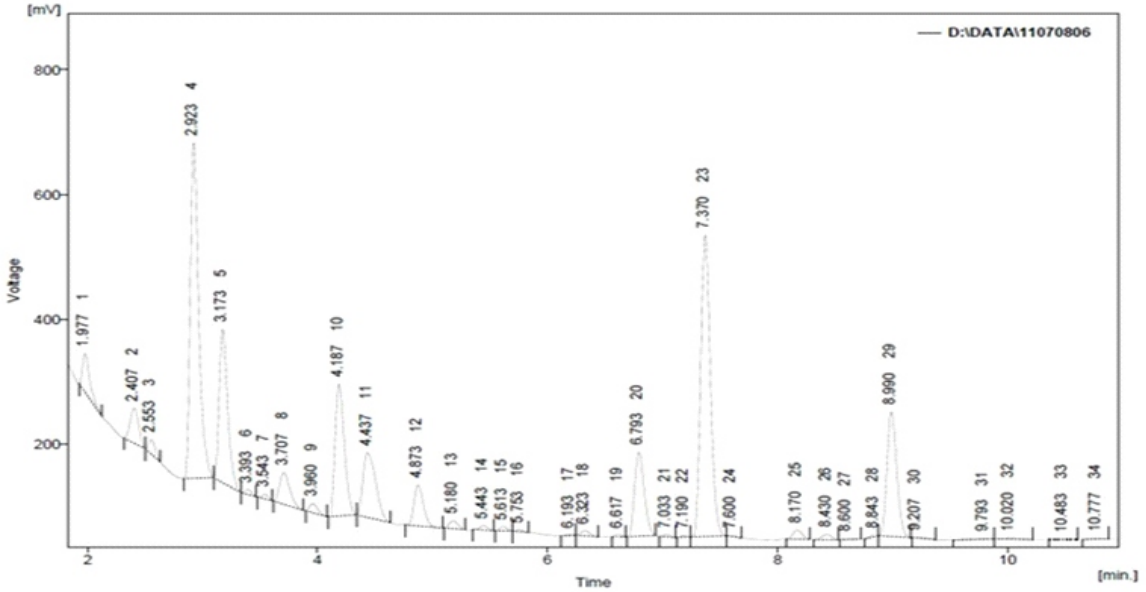


Figure 5: GLC tracing of the FAME of the TL of body flesh, of the crab, *Uca acuta acuta.* GLC column used was BPX-70 (Polar column) Megabore column (30 meters length x 0.530 mm dia).

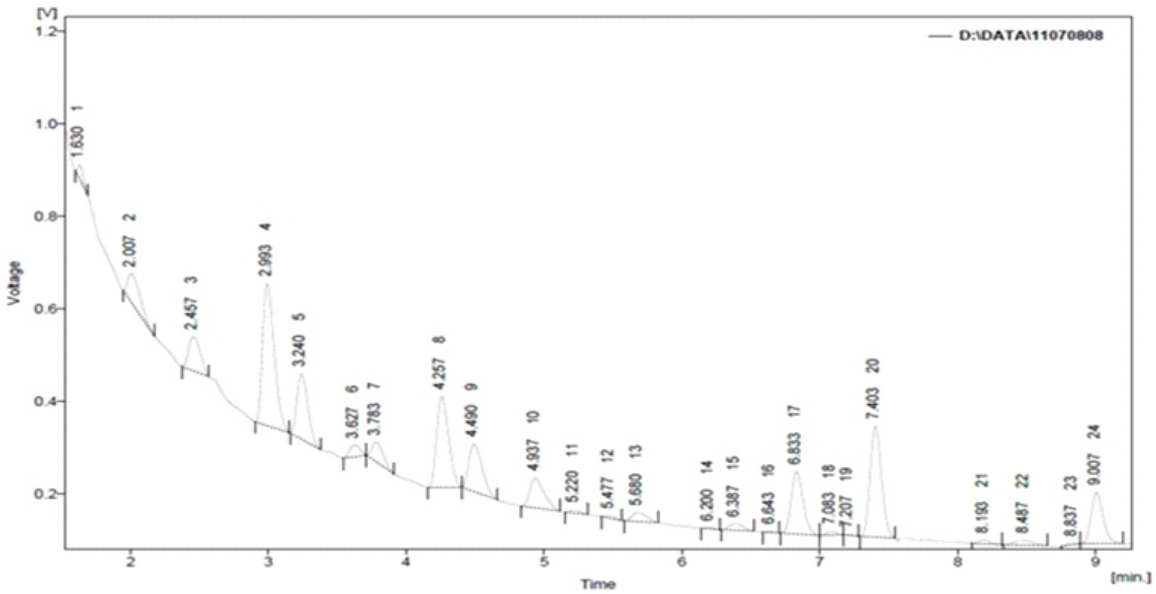


Figure 6: GLC tracing of the FAME of the TL of big chela flesh, of the crab, *Uca acuta acuta.* GLC column used was BPX-70 (Polar column) Megabore column (30 meters length x 0.530 mm dia).

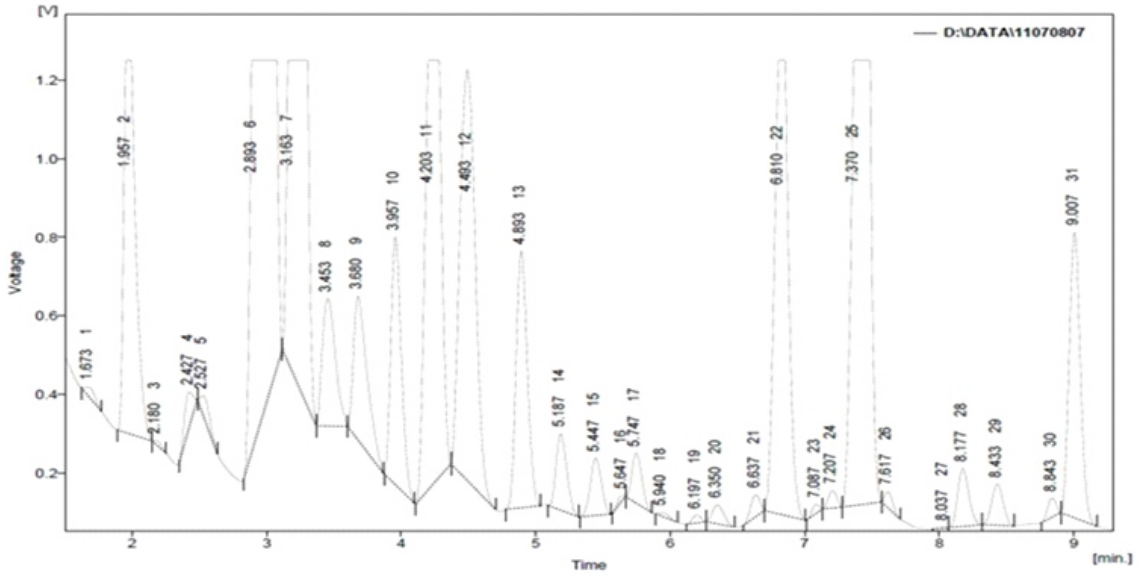
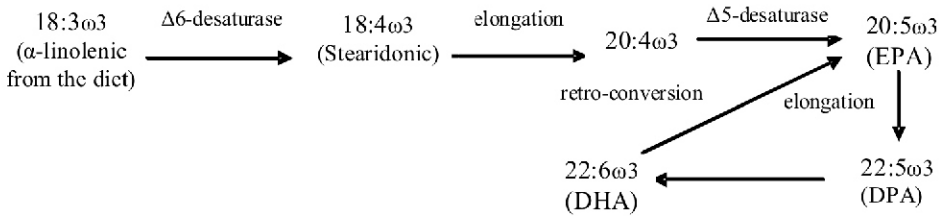


Figure 7: GLC tracing tracing of the FAME of the TL of hepatopancreas, of the crab, *Uca acuta acuta*. GLC column used was BPX-70 (Polar column) Megabore column (30 meters length x 0.530 mm dia).

chela flesh and it was 13.3% in the hepatopancreas . The EPA is the major bioactive fatty acid. Of the other polyenoic acids, special mention is to be made on DHA, which was found only at 9.3% in body flesh, 7.0% in big chela flesh and 4.8% in the hepatopancreas.

DISCUSSION

Omega-3 fatty acids, the principal building blocks of marine fish oils, have a number of health enhancing properties. Already well known for their ability to protect against heart disease, cancer, and diabetes,[22]the omega-3 fatty acids eicosapentaenoic



acid (EPA) and docosahexaenoic acid (DHA) may be highly effective in preventing and managing depression and cognitive decline, according to a considerable number of evidence[23, 24, 25]. The results have revealed the occurrence of considerably high levels of long chain polyunsaturated fatty acids (PUFA) of ω3 series in the various organs of the animal. Of the ω3 PUFA recorded in this study, mention can be made of eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3), which are known to have various physiological and therapeutic effects[26, 27, 28].In the present study, α-linolenic acid (18:3ω3) has been found in considerable amount (about 30-40%) in the leaf lipids of major plants of this area.α- linolenic acid (ALA, 18:3ω3) is the primary precursor molecule for the ω3 family of fatty acids in animal tissues[4, 20, 29], which must come from the diet i.e., from the plant leaves. On the other hand, the detritus contained only 2.7% of ALA. This signifies that the leaf litters exposed to tidal waters and microbial activities and thus breakdown of mangrove leaves is brought about[30-31]. Since only a small portion (5%) of the leaf material was found to have been removed by grazing insects before leaf abscission, most of the materials became widely dispersed by seasonal currents. A key group of small animals, comprising only a few species but very large numbers of individuals, (in the present study *Uca acuta acuta* being one) ingest large quantities of the vascular plant detritus, and thus participates in the food chain. However, in the present study it is clear that the *Uca acuta acuta* species consumes considerable amount of ALA (18:3ω3), by grazing on the detritus.The main pathways to the formation of eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) requires a sequence of chain elongation and desaturation steps (Δ5 and Δ6 desaturases) with acyl-coenzyme-A esters as substrates[32]. Thus α-linolenic acid is elongated and desaturated with double bonds being inserted between existing double bonds and carboxyl group. Also, retro-conversion of 22:6ω3 to 20:5ω3 may take place in the peroxisomes of the cells by removal of the first two carbon atoms by a process of β-oxidation (removal of two carbon units) under circumstances, as shown below:

The offspring of monkeys fed an n-3 PUFA deficient diet during pregnancy show visual impairments[33]. Supplementation of the infant monkeys with α-linolenic acid resulted in an increase in the concentration of DHA in neural tissues and an improvement in visual function[34]. The effect of α-linolenic acid deficiency on neurological function supports the role of α-linolenic acid as a precursor to longer chain n-3 PUFA which are critical in the function of the central nervous system[35]. Fifty percent of children and 30% of adults receiving long-term total parenteral nutrition lacking α-linolenic acid exhibited visual dysfunction, which suggests decreased availability of DHA for incorporation into neural membranes[36].

Only fragmentary studies have so far been made on the transmission of plant biochemicals to detritivore animals and their bio-conversion there upon. Of the studies so far done,in the Sunderbans mangrove forest, mention can be made of the fatty acids and sterols of mangrove leaves[37], triterpenoids and sterols of mangrove plant leaves[38]and fatty acids of the detritivores, *Boleophthalmus bodderti*[39]and biotransformation of oleanolic acid to oleanonic acid of mangrove leaves[40]. Some works on the lipids, fatty acids, sterols and triterpenoids have been done on the mangrove plant leaves and faunal components like gastropods, mudskippers of Sundarbans mangrove forest, India, but so far no such data has been generated in the mangrove patches and their associated benthic fauna of Midnapore (East). From the stand point of ecology, conservation and utilization of the natural wealth of mangrove, the present study has appeared to generate research information of immense ecological impact.

CONCLUSION

The present study indicates that:

1. The mangrove plant leaves from a selected study area in the coastal-estuarine tract of Midnapore(East),West Bengal,India have been found to contain high levels of α-linolenic acid (18:3ω3), which is a precursor of long chain PUFA's, viz., EPA (20:5ω3) and DHA (22:6ω3).

2. *Uca acuta acuta,* being an important intertidal bio-energetically significant macrobenthic fauna lives on leaf detritus as detritivores and thus consumes considerable amount of α-linolenic acid (ALA, 18:3ω3).

3. The ALA thus consumed by this abundant detritivore animal in the studied estuarine mud flat are converted *in vivo* to long chain polyunsaturated fatty acids of ω3 series, viz., EPA and DHA.

4. Considerably high levels of EPA and DHA have been found in the various tissues of *Uca acuta acuta*, particularly in the body flesh of the animal.

5. It has also been established that, *Uca acuta acuta* are capable of biosynthesizing long chain PUFA's efficiently based on the ingradients it consume from the mangrove leaf litter.

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