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Biotransformation of fatty acids by *Cerebratulus* sp. - A study from the eastern coast of India

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ABSTRACT

The present study deals with the fractional analysis of lipid components and their biotransformation process especially of -ω3 fatty acids from primary food sources(detritus, mangrove leaves and planktons) of *Cerebratulus* sp. - an intertidal benthic nemertine species found in the intertidal belt at the confluence of Subarnarekha estuary with the Bay of Bengal of West Bengal- Odisha coast, India. The primary food sources of this intertidal benthic fauna under study has been shown to contain appreciable amount of -ω3 fatty acids, particularly α- linolenic acid (ALA, 18:3ω3) which has been appeared to have been consumed and biotransformed in the process of food chain and food web interactions in the studied mangrove- estuarine ecosystem. On analyzing the fatty acids of muscles of studied species remarkable amount of 20:5ω3(EPA, Eicosapentaenoic acid) and 22:6ω3(DHA, Docosahexaenoic acid) have been found to remain present in the muscles of this animal. From the standpoint of biochemical ecology, these animals are being considered as a facilitator of chain elongation and simultaneous desaturation of ALA in order to produce essential long chain PUFA's (Polyunsaturated fatty acids), EPA and DHA which are now being recommended as an important pharmaceutical supplement.

Introduction

Mangrove ecosystem, a unique, fragile, highly productive ecosystem in the sea-land interphase, is the conglomerations of plants, animals and microorganisms acclimatized in the fluctuating environment of tropical intertidal zone. Trophic interactions in mangrove estuaries are often complex, largely because of the interactions of an array of ecological parameters both living and non living pertaining to both aquatic and forest subsystems(Chakraborty, 2011 and Samanta *et al* ., 2014).Out of several applicabilities the use of fatty acids as biomarkers to understand tropic interactions within estuarine ecosystems has been a reliable method for determining biotransformation and bioconversion strategies of benthic species at estuarine ecotone. In marine ecosystem, lipids provide the densest form of energy which is transferred from algae to vertebrates via zooplankton (Parrish, 2013). As, well they contain essential fatty acids and sterols which are considered to be important drivers of ecosystem health and stability.

Biochemical analysis of gut contents can help to studies the feeding behaviour and nature of fatty acids derived from their diet. The lipid components are not selectively processed during food intake and incorporation, and hence in many circumstances are integrally and markedly transferred through aquatic food webs (Dalsgaard, 2003). Thus fractional composition of fatty acids is being used to follow the transfer of energy through a marine food web which in turn enables qualitative assessment of the relative trophic position of an organism (Falk-Petersen, 2004).

Mangrove ecosystem dynamics contribute important energy transfers and have stronger trophic linkages with benthic invertebrates (Chakraborty, 2011). The benthic nemertine species *Cerebratulus* sp. has been reported from the intertidal mudflats of Subarnarekha estuary with Bay of Bengal which contributes valuable ecological services in respect of biomass and its bioturbatory activities (Ghorai *et al*., 2014).

The polyunsaturated fatty acids of –ω series cannot be synthesized by humans and are thus obtained through diet (Colombo *et al*., 2006). Although medical and nutritional importance of these fatty acid components have been established,(Colombo *et al*., 2006). Very few studies have been undertaken on efficient exploitation of these natural bioactive compounds. At present, marine fishes and fish oils are the main commercial sources of PUFAs but their suitability for human consumption has been questioned from a biosafety perspective, raising the need to search for alternative sources of high quality PUFAs (Bhosale *et al.*, 2008). Furthermore, the presence of chemical contaminants (e.g. mercury) in fish oil can be harmful to consumers (Adarme-Vega, 2012).

In such context, the present study on the food habits, biochemical analysis of specific lipid and fatty acid components from primary food sources to the different body parts of studied intertidal benthic animal, *Cerebratulus* sp., in a mangrove-estuarine ecosystem has been undertaken. Emphasis was also given to elucidate the spectrum of biotransformation and trophic relationships on one hand and also to recognize *Cerebratulus* as the dietary source of EPA and DHA on the other.

Materials and methods

Collection of biological samples

Mangrove leaves, detritus and *Cerebratulus* were collected from three contrasting study sites (namely SI, SII, and SIII) from the confluence of Subarnarekha estuary at Talsri (Longitude 87º5′ E to 88º5′ E and latitude 20º30′ N to 22º2′ N) near New Digha, West Bengal, India. Both phytoplankton and zooplankton were collected by filtering 100 liters of water using plankton nets having a mesh size of 0.35µ and 0.50µ respectively. The plankton samples, detritus, plant leaves and muscles (separated by dissection in the laboratory) of studied species were immediately frozen and stored at -20°C until analyzed.

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Analysis of fatty acids

Total lipids were extracted from each sample following Bligh and Dryer (1959) method. Identification and confirmation of fatty acids were done 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.

Table 1. Percentage of Total Lipid (TL) obtained from muscles of *Cerebratulus sp.* and its primary food sources.

Sample	Amount taken	Total lipid obtained	Percentage of total lipid(w/w)
Muscles	5.23 gm.	125.34 mg.	2.39
Planktons	3.81 gm	24.73 mg.	0.64
Mangrove leaves	15.22 gm	29.34 mg	0.19
Detritus	8.92 gm	16.39 mg.	0.18

Table 2. Fatty acid compositions of Total Lipids (TL) of muscles of *Cerebratulus* sp. and its primary food sources (Plankta, mangrove leaves and detritus) as determined by GLC of methyl esters (% w/w of each component in total fatty acids)

Components[a]	Muscles	Planktons	Mangrove leaves	Detritus
14:0	1.00	9.0	2.1	6.5
15:0	1.26	1.3	4.2	2.4
16:0	7.96	20.4	87.0	38.2
17:0	1.71	1.1	2.1	0.6
18:0	5.32	9.5	13.5	4.4
20:0	2.01			
21:0	0.27			
22:0	0.62	0.3	0.2	0.4
23:0	0.21			
24:0	0.13	1.3	0.4	0.7
Total SAFA	20.49	42.9	109.5	53.2
14:1	0.86	0.3		2.7
15:1	0.20	0.1	0.8	0.6
16:1	1.66	10.8	4.6	11.0
17:1	0.80	1.7	0.7	0.3
20:1	0.29			
18:1ω9	3.02	5.3	29.8	13.20
22:1	0.57	0.2		0.1
22:1ω9	0.57			
24:1	0.66	0.2		
TotalMUFA	8.63	18.6	35.9	27.9
16:2		0.2	0.4	1.1
17:2		2.2		
20:2	0.30			
22:2	0.43			
18:2ω6	1.84	1.9	49.50	9.0
18:3ω6	0.46	0.3	1.4	1.5
18:3ω3	1.36	3.1	100.70	2.7
20:3ω3	4.48	0.1		0.3
20:3ω6	0.00			
20:4ω6	5.00	2.6		0.2
20:5ω3	0.52	12.5		1.2
22:6ω3	2.89	11.1		0.2
Total PUFA	17.28	34	152	16.2
Total –ω3	9.25	26.8	100.7	4.4
Total –ω6	7.3	4.8	50.9	10.7
PUFA/SAFA	0.84	0.79	1.39	0.30

[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.

Results

Analysis of muscles and primary food sources of *Cerebratulus* sp.

Analysis of lipid contents of muscles and food sources of *Cerebratulus* sp have revealed that the muscles of *Cerebratulus sp.* contained highest amount (2.39%) of total lipids (TL) while detritus exhibited lowest amount (0.18%) as summarized in Table- 1 .

Fractional analysis of total lipids of muscles of *Cerebratulus* sp. and its primary food sources

Fractional components of fatty acids as represented in Table- 2 of TL showed that food components of *Cerebratulus sp.* included appreciable amount of α- linolenic acid (ALA). Muscles of *Cerebratulus* sp. have shown to contain 9 different types of MUFAs and 12 different types of PUFAs. Among PUFAs arachidonic acid (AA, 20:4ω6) registered highest amount (5.00%) followed by eicosatrienoic acid (ETE, 20:3ω3) 4.48% , docosahexaenoic acid (DHA, 22:6ω3) 2.89% and so on. However, mangrove leaves did not reveal the occurrence of AA, ETE and DHA . The quantity of linoleic acid (LA, 18:2ω6) exhibited moderate to high amount (1.84% - 49.50%) in different studied samples.

Discussion

Fatty acids have been used as qualitative markers to trace or confirm predator-prey relationships in the marine environment for more than thirty years (Prato *et al*., 2012). More recently, they have also been used to identify key processes impacting the dynamics of some of the world's major ecosystems. The fatty acid trophic marker concept is based on the observations that marine primary producers lay down certain fatty acids which may, be transferred conservatively to, and hence can be recognized in, primary consumers (Dalsgaard *et al*., 2003).

In a marine ecosystem, generally qualitative similarities are observed in the fatty acid composition of the organisms which occupy different trophic levels. The first link of the food chain i.e. phytoplankton are able to synthesize all the fatty acids de novo and composition of fatty acids changed significantly in the decomposing leaves of mangroves (Moreno *et al*., 1979 and Alikunhi *et al*., 2010). High concentration of -ω3 fatty acids which are generally considered as to be fatty acids of marine life, were found to have been mainly contributed by some phytoplanktonic species (diatoms, dianoflagellates etc.) to the marine ecosystem (Sargent *et al*., 1976). Examination on muscles of *Cerebratulus* sp., an intertidal detritivorous macrobenthic nemertine of the studied areas revealed the presence of appreciably high amount of 20:5ω3 fatty acids (Table- 2) associated with other polyunsaturated fatty acids of the –ω3 series. It is thus envisaged that intake of higher ammount of the precursor acid (18:3ω3), through primary food sources and their subsequent chain elongations and desaturation processess de novo would lead to the formation of –ω3 unsaturated fatty acids in higher levels. Intake of these diets enriched with –ω3 acids may explain the mode of accumulation of these PUFAs in variable amounts in the body parts (muscles) of studied macrobenthic estuarine fauna.

The α-linolenic acid (ALA, 18: 3ω3), the primary precursor molecule for the –ω3 family of fatty acids (Gunstone, 2002) in animal tissues must come from diet. The principal pathways to the formation of eicosapentaenoic acid (EPA, 20:5ω3) and decosahexaenoic acid (DHA, 22:6ω3) require a sequence of chain elongation and desaturation steps (Δ5 and Δ6 desaturases) with acyl-coenzyme-A esters as substrates (Vance and Vance, 2008). The bioconversion of ALA in the present studied faunal component is supposed to occur as-

$$18{:}3\omega3 \xrightarrow{[+2C]} 20{:}3\omega3 \xrightarrow{[-2H]} 20{:}4\omega3 \xrightarrow{[-2H]} 20{:}5\omega3 \xrightarrow{[+2C]} \text{(EPA)}\ 22{:}5\omega3 \xrightarrow{[-2H]} 22{:}6\omega3\ \text{(DHA)}$$

starting from linolinate (18:3ω3) obtained by the animal from their primary food sources which have been also supported by San Giovanni and Chew, 2005 and Kapoor and Patil, 2011.

Fatty acids are useful tool to understand trophic ecology and determination of food web connections, contrary to more traditional gut content analysis which provide information on dietary intake and food constituents leading to the sequestration of lipid reserves over a longer period of time (Auel *et al*., 2002). The presence of EPA and DHA within muscles of studied nemertine has elucidated the fact that these pharmaceutically important fatty acids are thought to have been derived from ALA, present in diets of *Cerebratulus sp.* through biotransformation processess within the body of studied fauna. All marine animals including invertebrates, remarkably accumulate various sorts of *n*-3 PUFA in their lipids, in particular docosahexaenoic acid (DHA, 22:6*n*-3) and eicosapentaenoic acid 6 (EPA, 20:5*n*-3), while *n*-6 PUFA are generally observed as the major fatty acids in terrestrial animal lipids (Saito and Aono 2013)

Low values of the PUFA/SAFA ratio (Tables- 2) as determined in the present research investigation are because of the presence of higher levels of palmitic acid (16:0), suggesting a contribution of vegetal detritus in the diet of *Cerebratulus* sp. PUFAs in green algae predominantly comprised of 18:2ω6 and 18:3ω3 and these fatty acid compositions are similar to those of terrestrial (Vascular) plants since they have common ancestors (Raven *et al*., 1992). In the present study, an appreciable amount of 18:2ω6 and 18:3ω3 have been estimated (Tables-2) indicating that the species under study used to consume considerable amount of green algae (phytoplankton) occurring over the surface of the soil and also from the supply of neighboring mangrove vegetations. Terrestrial organic matters can also be associated with bacteria or fungi and constitutes an attractive and energetically utilizable food sources for invertebrates (Barlocher and Corkum, 2003). In the present study, the odd branched fatty acids have been recorded as an indicator of bacterial derivative which highlights a source of food supply for *Cerebratulus* from decaying organic matters. Presence of highest amount of AA (20:4ω6, Table- 2) in the muscles of studied species further indicated that detritus serves as one part of food of *Cerebratulus*. Presence of moderate to high levels of EPA and DHA, within muscles of studied species, derived through bioconversion of ALA from food sources indicated that they have been the good sources of EPA and DHA which are being considered as the precursors of several metabolites that are potent lipid mediators. Many investigators recognize them as to be the beneficial components for human being as in the prevention or treatment of several diseases (Serhan *et al*., 2008).

Lipid has been recognized as essential component in animal nutrition as well as aquaculture feed. Therefore, deposition of lipid (Fatty acid) which found as a major constituent in methanolic extract of *cerebratulus* sp. might be obtained from their food. These compounds help to defend *Cerebratulus* sp. against predators. The invented chemical defenses are thought to provide ecological advantages and may function as a driving force in the evolution of this group (Cimino and Ghiselin, 1998 and Prachi *et al.*, 2012). Present investigation has revealed that muscles of the studied animal stored major amount of all fatty acids and presence of EPA and DHA in plankton samples indicated that *Cerebratulus* obtains these fatty acids from the planktons as food source. Major mangrove plant leaves of the studied area have been found to possess moderate to high amount of α-linolenic acid (18:3ω3) which is the precursor of long chain PUFAs viz. EPA (20:5ω3) and DHA (22:6ω3). Presence of high levels of carnivorous markers of the studied species i.e. oleic acid (18:1ω9, derived from animal sources because of the consumption of zooplankton, animal detritus etc, occurred in the intertidal belts) in muscles of *Cerebratulus* have indicated that they are the inhabitants of the studied ecotone. Presence of high amount of 22:6ω3 and 20:4ω6 in animal samples has established the facts that diatoms, dianoflagellates and macroalgae constitute the basal portion of food pyramid of this complex estuarine ecosystem.

The Eicosatrienoic acid has been reported to induce spawning in the male lugworm, *Arenicola marina* (Pacey and Bentley 1992). The heighest amount ETE among all detected fatty acids during present investigation indicated the fact that this particular FA is thought to play important role in their reproductive strategy during breeding period. The ability of PUFAs, particularly GLA (γ-linolenic acid), recorded during present FA analysis has tended to enhance free radical generation and lipid peroxidation process specifically in tumor cells which is supposed to be because of their tumoricidal actions (Das, 2004 and Kirubakaran *et al*., 2011).

Conclusion

The present investigation has highlighted the mode of occurrence of different classes of fatty acids in body muscles of a mangrove- estuarine benthic macrofauna, *Cerebratulus* sp. inhabiting in an ecotone, at the confluence of an estuary (Subarnarekha) with Bay of Bengal (longitude 87°5'E to 88°5'E and latitude 20°30'N to 22°2'N) in the North- East coast of India. Estimation of such biochemical entities has also been made from the habitat of the studied species, especially its food sources (mangrove leaves, planktons and detritus).The variabilities in the amount of different fatty acid components in the muscles of studied species and its food sources have prompted to arrive at a conclusion on the mode of biotransformations and bioconversions of these bioactive substances.

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