Fatty Acids as Tracer of Trophic Relationships in a Subtropical Mangrove Wetland†

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ABSTRACT : To elucidate the trophic relationships within a subtropical wetland, the profile of fatty acids in producers, consumers and sediments of the mangrove forest and intertidal mudflat was studied. Results indicated a close relationship in fatty acid profiles between the mangrove plants *Aegiceras corniculatum* and *Avicennia marina* and the sesarmid crab *Sesarma bidens*, and between the fiddler crab *Uca arcuata* and diatoms. The fatty acid profile of the mudskippers *Boleophthalmus pectinirostris* and *Periophthalmus cantonensis*, however, showed a mixed diet of diatoms, macroalgae, protozoa and crabs. Seasonal changes in fatty acid profiles in mangrove plants, sediments and sesarmid crabs were noted. The implication of using fatty acids as tracer of trophic relationships was discussed.

Key words : Fatty acids, Trophic relationship, Wetland

INTRODUCTION

Trophic relationships in mangrove wetland are complex. In addition to export of organic matters from mangrove forest to near-shore areas, there is *in-situ* consumption and nutrient recycling among various trophic levels within the ecosystem. Recent research suggested that different primary producers possess unique fatty acid markers that could be used as specific tracers for determining trophic relationships (Kharlamenko *et al*. 2001). For examples, fatty acid 16:1 (n-7) is a marker for diatoms (Ackman *et al*. 1968), 22:6 (n-3) for zooplanktons, and 20:5 (n-3) for diatoms and algae (Kayama *et al*. 1989). Fatty acids in sediment were also analyzed and used to study the distribution of microorganisms (Volkman *et al*. 1980). Meziane *et al*. (1997) attempted to use lipid markers to define the sources of organic matter in sediment and food web from a wetland. The present study investigated the trophic relationships within a tropical wetland using fatty acids as a tracer and assessed the validity of the results.

MATERIALS AND METHODS

Samples of senescent leaves of mangrove plants *Aegiceras corniculatum* and *Avicennia marina* were picked from tree branches at the Mai Po wetland on the northwest of Hong Kong (24°30、N, 114°01、E). Macroalgae were collected by scrapping the biomass from mudflat surface whereas benthic diatoms were sampled using the lens tissue method, in which lens tissue papers were laid on the mudflat surface at day time during low tide, and diatoms were attracted and attached onto the papers through phototactic response. Specimens of different consumers, including the sesarmid crab *Sesarma bidens*, fiddler crab *Uca arcuata* and mudskippers, *Boleophthalmus pectinirostris* and *Periophthalmus cantonensis* were collected by trapping or using a fish net. Sediment samples from the mangrove forest were obtained using a shovel. Samples were collected both in summer and winter whenever possible, except for the mudskipper *B. pectinirostris* which was obtained in summer and diatoms, microalgae and the mudskipper *P. cantonensis* which were captured in winter only.

For fatty acid analysis, procedures modified from Bligh and Dyer (1959) was followed. Lipids from 1 g dry weight of plant, 250 mg animal tissue or 10 g sediment were extracted by chloroform-methanol, followed by washing with 0.04% $CaCl_2$ solution, centrifugation and removal of the aqueous layer. The remaining organic layer was rinsed twice with 0.02% $CaCl_2$ solution in methanol, and evaporated under nitrogen. Ice-cold acetone was added and further dried under nitrogen. The resultant solution was methylated with 6% sulphuric acid in methanol and incubated. After cooling, double-deionized water and petroleum ether were added and the upper petroleum ether phase was transferred to a vial and dried by nitrogen. The dried extract was diluted by hexane and fatty acid methyl esters (FAMEs) were determined using gas chromatography equipped with flame ionization detector. Internal standards of known FAMEs were used for validation of the chromatograph peaks.

† This article was presented at the INTECOL meeting (Seoul, August 2002).

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Table 1. Fatty acid profile (mean % dry weight) of primary producers, consumers and sediments in summer of a subtropical wetland (n=5)

FAMEs	*Aegicera*	*Avicennia*	*Sesarma*	*Uca*	*Boleophthalmus*	Sediment
8:0	0.0	0.0	0.0	0.0	0.0	1.1
9:0	0.0	0.0	0.0	0.0	0.0	0.0
10:0	0.2	0.0	0.0	0.0	0.0	1.3
11:0	0.0	0.0	0.0	0.0	0.0	2.1
12:0	1.3	0.3	0.3	0.0	0.2	3.3
13:0	0.0	0.0	0.2	0.1	0.6	3.1
14:0	1.8	3.6	1.6	2.1	3.4	9.5
14:1	0.0	0.0	0.0	0.0	0.0	4.5
15:0	0.0	0.1	1.1	2.6	3.3	2.3
16:0	10.8	12.0	15.5	12.9	19.6	21.2
16:1(n-9)	0.0	0.9	7.6	10.3	5.9	6.3
17:0	0.8	0.8	0.6	2.9	3.9	3.2
17:1(n-9)	0.0	0.0	0.0	0.0	0.0	0.0
17:1(n-10)	0.0	0.0	0.0	0.0	0.0	0.0
18:0	2.2	3.84	2.9	6.7	10.9	5.8
18:1(n-6 *cis*)	0.0	1.23	0.0	0.0	0.0	0.0
18:1(n-9 *cis*)	0.0	0.0	0.0	0.0	0.0	0.0
18:1(n-9 *trans*)	10.9	6.6	23.5	10.5	7.6	6.8
18:1(n-11 *cis*)	0.5	0.2	1.9	4.1	4.5	5.5
18:2	0.0	0.0	0.2	0.0	0.2	0.9
18:2(n-9,12 *trans*)	18.5	13.6	13.9	4.2	1.7	11.2
18:3(n-6,9,12)	0.0	0.0	0.0	1.0	0.8	0.0
18:3(n-9,12,15)	51.7	48.0	6.4	0.7	0.6	8.4
19:0	0.2	0.3	0.6	0.0	0.0	0.0
20:0	0.0	0.0	0.9	0.0	0.8	1.0
20:2	0.2	0.0	0.8	0.8	0.4	0.0
20:4(n-5,8,11,14)	0.7	0.0	3.3	7.9	9.2	0.4
20:5(n-5,8,11,14,17 *cis*)	0.0	0.0	3.9	18.2	16.1	0.2
21:0	0.0	0.5	0.6	0.8	0.0	0.0
22:0	0.2	1.2	1.4	1.2	1.1	1.1
22:1(n-13 *cis*)	0.0	0.0	0.0	0.0	0.0	0.0
22:2	0.0	0.0	0.0	0.0	0.0	0.1
22:4	0.0	0.1	0.0	0.0	0.0	0.0
22:6	0.0	0.0	2.7	13.0	6.0	0.0
23:0	0.0	0.0	1.3	0.2	2.7	0.2
24:0	0.0	6.9	0.6	0.0	6.6	0.5
24:1(n-15 *cis*)	0.0	0.0	0.0	0.0	0.0	0.0
25:0	0.0	0.0	0.3	0.0	0.0	0.0
26:0	0.0	0.0	0.4	0.0	0.0	0.2
27:0	0.0	0.0	0.0	0.0	0.0	0.0
28:0	0.0	0.0	0.0	0.0	0.0	0.0
30:0	0.0	0.0	0.0	0.0	0.0	0.0

FAMEs = fatty acid methyl esters.

x:y (n-z), where x = number of carbon atoms, y = number of double bonds, n-z = position of double bond from the terminal methyl group.

Table 2. Fatty acid profile (mean % dry weight) of primary producers, consumers and sediments in winter of a subtropical wetland (n=5)

FAMEs	*Aegicera*	*Avicennia*	*Sesarma*	*Uca*	*Perio-phthalmus*	Sediment	Micro-algae	Diatoms
8:0	0.0	0.0	0.0	0.0	0.0	1.15	0.0	0.0
9:0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
10:0	0.4	0.0	0.3	0.0	0.0	1.3	0.2	0.2
11:0	0.2	0.0	0.1	0.0	0.0	1.8	0.0	0.0
12:0	1.8	0.3	0.4	0.2	0.5	3.4	0.5	0.2
13:0	0.0	0.0	0.0	0.18	0.2	2.4	1.1	0.4
14:0	1.7	3.1	2.7	3.0	3.3	7.0	2.6	7.2
14:1	0.8	1.3	0.3	0.1	0.3	3.5	0.2	0.3
15:0	0.1	0.2	1.4	1.6	0.7	3.9	0.8	4.8
16:0	13.4	16.6	16.7	12.9	18.5	17.9	22.9	15.1
16:1(n-9)	0.8	1.0	6.2	8.2	6.9	7.7	31.2	14.9
17:0	1.0	0.9	1.0	3.6	1.8	5.0	0.7	10.2
17:1(n-9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17:1(n-10)	0.0	0.0	0.0	0.0	0.0	1.8	0.0	4.1
18:0	2.4	5.0	2.8	4.3	5.3	6.8	1.1	0.6
18:1(n-6 *cis*)	0.0	0.0	1.4	0.3	0.0	0.0	0.0	0.0
18:1(n-9 *cis*)	0.0	0.0	0.3	0.0	0.0	1.8	0.0	0.3
18:1(n-9 *trans*)	20.0	14.1	28.6	11.3	15.9	9.5	8.1	1.6
18:1(n-11 *cis*)	0.6	0.4	2.6	4.0	5.2	4.1	1.3	0.8
18:2	0.0	0.0	0.2	0.3	0.5	1.5	0.0	0.0
18:2(n-9,12 *trans*)	27.3	13.8	15.3	4.3	6.2	7.0	3.1	1.6
18:3(n-6,9,12)	0.0	0.0	0.0	0.0	0.0	1.2	0.2	0.4
18:3(n-9,12,15)	24.7	31.6	10.1	0.9	1.2	3.0	0.2	3.9
19:0	0.0	0.7	0.7	0.5	0.6	2.0	0.2	0.0
20:0	0.5	4.4	0.4	1.7	0.8	1.1	0.0	5.8
20:2	0.0	0.0	0.0	0.6	0.9	1.1	0.3	0.1
20:4(n-5,8,11,14)	1.9	0.0	1.8	8.5	13.9	0.6	3.9	2.4
20:5(n-5,8,11,14,17 *cis*)	0.0	0.0	2.3	23.2	7.6	0.4	21.1	20.2
21:0	0.2	1.0	0.4	0.0	0.0	0.9	0.0	0.0
22:0	0.8	2.4	1.1	1.1	0.0	1.3	0.1	0.0
22:1(n-13 *cis*)	0.0	0.0	0.9	0.3	0.0	0.3	0.0	0.7
22:2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:4	0.0	0.0	0.2	7.8	4.2	0.0	0.0	0.3
22:6	0.0	0.0	1.4	0.6	4.6	0.0	0.2	3.8
23:0	0.3	1.0	0.4	0.3	0.0	0.3	0.1	0.0
24:0	0.8	2.5	0.3	0.0	0.6	0.0	0.1	0.0
24:1(n-15 *cis*)	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
25:0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
26:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

FAMEs = fatty acid methyl esters.

x:y (n-z), where x = number of carbon atoms, y = number of double bonds, n-z = position of double bond from the terminal methyl group Legend for figure.

To compare seasonal variations in fatty acid profiles, data of 42 FAMEs in the biological and sediment samples recorded in both summer and winter were subjected to principal component analysis (PCA) (Tabachnick and Fidell 1996).

RESULTS

Table 1 and 2 show the fatty acid profile quantified for the biological and sediment samples collected in summer and winter, respectively, in this study. Results of the fatty acid profiles indicated that the mangrove plants *Aegiceras corniculatum* and *Avicennia marina* contained a high content (59.5~81.1% dry weight) of 18:1 (n-9), 18:2 (n-9) and 18:3 (n-9) mono- and poly-unsaturated fatty acids. Such fatty acids (43.8~54.3% dry weight) were also identified in the sesarmid crab *Sesarma bidens* (Tables 1 & 2), suggesting that these mangrove leaves were the major food source for *S. bidens*. However, the fiddler crab *Uca arcuata* had a different fatty acid pattern, with high ratios of 0.64~0.80 for 16:1 (n-9) to 16:0 and 0.83~0.85 for C16 to C18 chains. Such high ratios reflected the diatom biomarker in their diets (Table 2). The two mudskippers *Boleophthalmus pectinirostris* and *Periophthalmus cantonensis* had relatively similar fatty acid profiles, with 61.2~63.4% of 16:0, 18:0, 18:1 (n-9), 20:4 (n-5) and 20:5 (n-5) acids (Tables 1 and 2). Such profiles were indicative of an omnivorous diet comprising diatoms, macroalgae, protozoa and crabs. The mangrove sediments primarily comprised of saturated fatty acids (55.0~70.9%) with C8-C26 chains. Seasonal changes in fatty acid profile in the mangrove plant *Avicennia marina*, sediments and sesarmid crab *Sesarma bidens* were noted, as shown by a wider separation of their summer and winter samples in the PCA plot along the second principal component (Fig. 1). Both components accounted for 54.8% of variation in the data set.

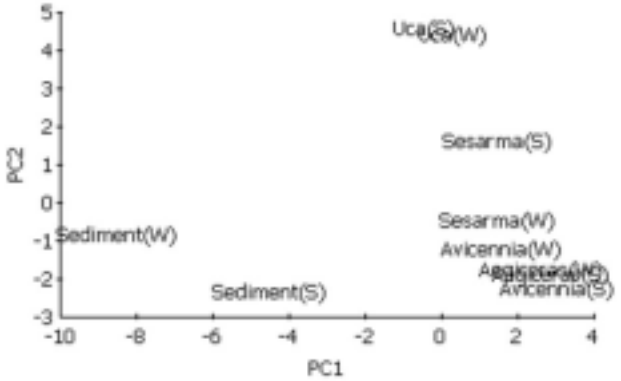

Fig. 1. A plot of results of principal component analysis of summer and winter fatty acid profile data (position of samples closer to each other indicates similar profile based on the 42 FAMEs, S = summer, W = winter, PC1 = first principal component, PC2 = second principal component)

DISCUSSION

The present study demonstrated that fatty acid profile can be a good marker for indicating various food sources. Studies have shown that fatty acids 16:1 (n-9)/16:0 and C16/C18 reflect food source from diatoms, 18:1 (n-9), 18:2 (n-9) and 18:3 (n-9) from mangrove leaves, 20:4 (n-5) from protozoa, and 20:5 (n-5) from algae; whereas 22:6 (n-4) showed a carnivorous feeding mode (Meziane and Tsuchiya 2000, Kharlamenko *et al*. 2001, Reuss and Poulsen 2002). Our data on comparison of fatty acid profiles of primary producers, consumers and sediments revealed that trophic relationships are closely linked to various habitats within the wetland ecosystem. As the sesarmid crabs live near mangrove stands, their fatty acid profile showed that they consume a large portion of mangrove leaves. Only vascular plants synthesize low chain fatty acids (Currie and Johns 1988), and thus could be used as markers for mangrove leaves on the shore sediment (Meziane and Tsuchiya 2000). The fiddler crabs live on mud surface on the intertidal flat. Their fatty acid profile thus showed that they feed on diatoms from the mudflat predominantly. Our results are similar to the findings by Meziane *et al*. (2002), in which diatoms were the major diet of *Uca vocans* because of its higher mobility for searching for food on the shore. The mudskippers build borrows in the mudflat and voraciously feed on a variety of prey, as reflected in their omnivorous feeding mode from their fatty acid profile. Meziane and Tsuchiya (2000) also demonstrated the usefulness of using fatty acids to trace organic matter cycling in sediment and food web in a mangrove swamp.

In this study, seasonal changes in fatty acid profiles in mangrove plants, sediments and sesarmid crabs were noted. From PCA results, there appeared differences in the proportion of saturated to unsaturated (especially for 16:1, 20:4, 20:5 and 22:6) fatty acids in the summer and winter samples of the same species. Such differences could be due to changes in nutrient recycling within the wetland ecosystem during the dry (winter) and wet (summer) seasons (Meziane and Tsuchiya 2000). In subtropical wetland, litter decomposition in summer is relatively faster than that in winter owing to a higher temperature and humid environment. This may enrich the mangrove sediment and provide a larger food source for the sesarmid crabs (Fleming *et al*. 1990). Thus, seasonal changes in fatty acid profiles of various trophic levels reflect the dynamic nature of availability of food sources within subtropical wetland.

ACKNOWLEDGEMENTS

We thanked Harry Chai, Ball Lam, Paul Chow and K.H. Ho for assistance in the field. The work described in this paper was fully

supported by a Direct Allocation Grant (Project No. 7100166) from the City University of Hong Kong.

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(*Received December 9, 2003; Accepted April 1, 2004*)