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Preliminary Report on Stable Isotope Ratio Analysis for Samples from Matang Mangrove Brackish Water Ecosystems

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Abstract

The stable carbon and nitrogen isotope ratios of mangrove leaves, particulate organic matter (POM), shrimps and crabs, squids and fishes were studied for tracing the actual food web structure in the Matang mangrove brackish water ecosystems. Carbon isotope ratio of POM showed a positive correlation with the level of salinity, suggesting that POM around the mouth to mudflat areas of the river may consist of phytoplankton-derived detritus from the sea, whereas POM from mid- to upstream areas of the river may consist of mangrove-derived detritus. $\delta^{13}C$-$\delta^{15}N$ map showed a C-N isotope ratio gradient with the lowest value with mean (±SD) of $\delta^{13}C$ (-28.74±0.031‰) and $\delta^{15}N$ (4.36±0.15‰) for a mangrove leaf (Rhizophora apiculata), the highest value of $\delta^{13}C$ (-20.45±0.026‰) for a squid (Loligo duvaucelii) and of $\delta^{15}N$ (13.63±0.021‰) for a fish (Stolephorus commersonii). Penaeid shrimps (Penaeus merguiensis, Metapenaeus brevicornis and M. ensis) and mangrove crabs (Scylla serrata) showed intermediate C-N values between those of mangrove leaves and fish/squid.

Discipline: Fisheries resources
Additional key words: mangrove litter, food-chain, energy flow

Introduction

Brackish waters occur where sea water and freshwater mix, usually within regions characterized by flat topography stretching between rivers and seas. In tropical and subtropical areas, brackish water coastlines often play host to mangrove forests, which act as nursery grounds for fish and prawns while playing critical roles in protecting coasts from the threat of hurricanes and erosion. Humans also utilize mangrove trees for fuel, charcoal, chips, timber and medicinal products.

Matang Mangrove Forest Reserve in Perak State, is reputed to be the world's best managed forest. This reserve consisting of some 40,711 ha of mainly *Rhizophora apiculata* mangroves is located on the northwestern coast of Peninsular Malaysia. It is the largest tract of mangrove forest in Peninsular Malaysia and has been under sustainable yield management since the early part of the century, consisting of many islands and adjacent numerous waterways (rivers, channels, inlets and streams). Among these waterways, Selinsing river (Sungai Selinsing in Malay language) is the least disturbed river, and Sangga river (Sungai Sangga) is a main ship course of otter-trawlers. Particularly around the mouth area of the river, there is a Chinese fishing village and many rafts are engaged in cage culture of finfish (mainly giant sea-bass and mangrove snapper). We

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collected samples from the above 2 rivers in Matang, to analyze the trophic relations in mangrove brackish water ecosystems.

Measurements of natural $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios are useful for determining sources of nutrition for consumers and trophic relationships among organisms and have often been used in studies of lake, river and marine food webs[2,4,6,11,14,16–18]. It is generally recognized that $\delta\ ^{13}C$ measurements primarily indicate the main sources of carbon for consumers, and $\delta\ ^{15}N$ values indicate the trophic distance of consumers from the food base[5]. The simultaneous use of $\delta\ ^{13}C$ and $\delta\ ^{15}N$ values therefore enables to analyze the trophic relationships in food webs[3].

Measurements of $\delta\ ^{13}C$ and $\delta\ ^{15}N$ values in mangrove brackish water ecosystems have been carried out by many researchers[1,3,7–10,12,13,15] who reported the presence of a $\delta\ ^{13}C$ gradient at the higher trophic levels.

Materials and methods

1) Sampling and processing

Samples for isotope analysis were collected from Sungai Selinsing and Sangga Besar Matang, in November 1996 and February 1997 (Fig. 1). Five species of shrimps, 4 mangrove crabs and 8 squids with different body sizes, and 7 species of fishes were collected from Sungai Selinsing. Leaves from 2 species of mangrove and 7 bottles of surface water for the isotope analysis of particulate organic matter at different stations from mouth/mudflat to upstream areas of the river were also sampled from Sungai Sangga (Table 1). The length and weight were measured for each sample, then fat/gut were removed and

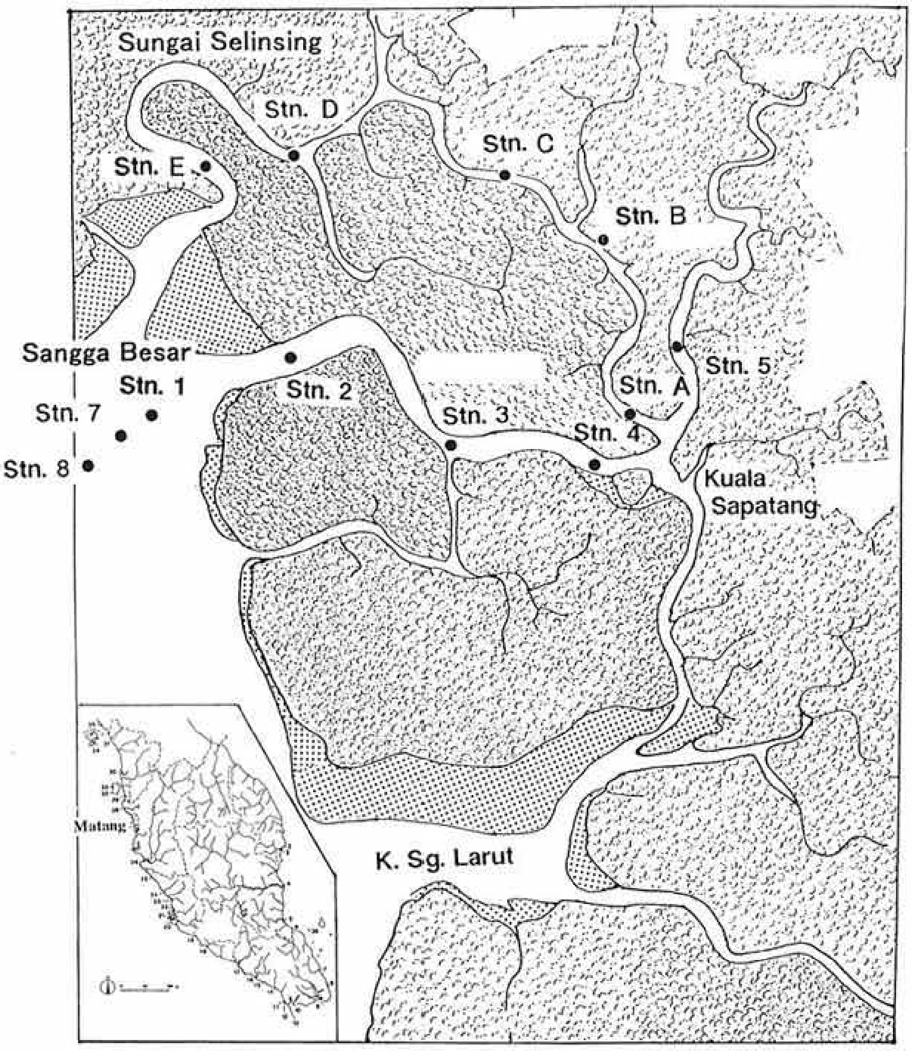

Fig. 1. Sampling stations for stable isotope analysis in Sungai Selinsing and Sangga Besar, Matang

Table 1. Sample specimens for stable isotope ratio analysis in the Matang mangrove brackish water ecosystem

1) Sungai Selinsing, Matang

Animal group	Japanese name	Date	Station	No.	Range (cm)	Range (g)	$\delta^{15}N$ (‰)	SD (‰)	$\delta^{13}C$ (‰)	SD (‰)	Remarks
Shrimps					**CL**[d]	**BW**[g]					
Penaeus merguiensis (L)[a]	Tenjikukurumaebi	Feb. 6, '97	Stns. A ~ E	5	2.4 ~ 2.8	5.48 ~ 8.51	8.910	0.023	−24.777	0.038	
P. merguiensis (M)[b]	Tenjikukurumaebi	Feb. 6, '97	Stns. A ~ E	5	2.0 ~ 2.3	4.32 ~ 5.38	8.640	0.048	−23.165	0.015	
P. merguiensis (S)[c]	Tenjikukurumaebi	Feb. 6, '97	Stns. A ~ E	5	1.2 ~ 1.8	1.22 ~ 1.92	9.765	0.025	−24.223	0.045	
Metapenaeus brevicornis	Yoshiebi zoku	Feb. 6, '97	Stns. A ~ E	7	1.2 ~ 1.6	0.95 ~ 1.77	8.359	0.033	−26.613	0.030	
M. ensis	Yoshiebi	Feb. 6, '97	Stns. A ~ E	7	1.5 ~ 1.9	1.51 ~ 2.38	8.778	0.023	−25.032	0.031	
Exhippolysmata ensirostris	Not available	Feb. 6, '97	Stns. A ~ E	8	0.8 ~ 1.2	0.36 ~ 0.86	10.284	0.034	−23.580	0.019	
Macrobrachium rosenbergii	Onitenagaebi	Feb. 6, '97	Stns. A ~ E	1	2.0	4.20	11.109	0.027	−24.111	0.034	
Mangrove crab					**CL**	**BW**					
Scylla serrata (L)	Nokogirigazami	Feb. 6, '97	Stns. A ~ E	1	10.2	290	10.563	0.033	−23.527	0.031	
S. serrata (M)	Nokogirigazami	Feb. 6, '97	Stns. A ~ E	1	8.1	110	9.800	0.017	−23.505	0.026	
S. serrata (S)	Nokogirigazami	Feb. 6, '97	Stns. A ~ E	2	6.8 ~ 7.1	60 ~ 80	8.891	0.240	−24.476	0.022	
Squid					**ML**[e]	**BW**					
Loligo duvaucelii (L)	Ajiajindouika	Feb. 6, '97	Stns. A ~ E	3	4.6 ~ 4.9	7.29 ~ 10.51	12.673	0.030	−20.855	0.010	
L. duvaucelii (S)	Ajiajindouika	Feb. 6, '97	Stns. A ~ E	5	2.8 ~ 4.0	2.01 ~ 4.61	13.182	0.016	−20.446	0.026	
Fishes					**SL**[f]	**BW**					**Gut contents**
Ambassis gymnocephalus	Takasagoishimochi zoku	Nov.16, '96	Stn. D	9	2.2 ~ 4.3	0.18 ~ 1.88	11.738	0.020	−24.057	0.022	Polychaeta
Anodontostoma chacunda	Nanyoukonoshiro	Nov.16, '96	Stns. D ~ E	19	3.3 ~ 6.6	0.54 ~ 3.95	9.727	0.022	−21.014	0.017	Diatom
Panchax melostigma	Not available	Nov.16, '96	Stn. D	10	2.0 ~ 2.4	0.08 ~ 0.18	12.256	0.022	−24.305	0.026	Phytoplankton
Stolephorus commersonii	Indoainokoiwashi zoku	Nov.16, '96	Stn. E	10	4.8 ~ 6.3	0.95 ~ 2.15	13.633	0.021	−25.008	0.026	Mysis
S. insularis	Indoainokoiwashi zoku	Nov.16, '96	Stn. E	6	4.9 ~ 6.8	0.98 ~ 2.90	13.454	0.025	−23.656	0.025	Mysis
Johnius voglerii	Konibe zoku	Nov.16, '96	Stn. E	4	11.1 ~ 11.9	24.9 ~ 35.3	12.377	0.015	−22.864	0.014	Mys.,Shr.,Fish
Lutjanus vitta	Tatefuedai	Nov.16, '96	Stn. E	1	11.1	30.6	11.897	0.017	−24.207	0.038	Shrimp

a): L; Large size. b): M; Middle size. c): S; Small size. d): CL; Carapace length. e): ML; Mantle length. f): SL; Standard length. g): BW; Body weight.

Table 1. (continued)

2) Sungai Sangga Besar, Matang

Leaves and POM	Japanese name	Date	Station	No.	Range (g)	δ ^{15}N (‰)	SD (‰)	δ ^{13}C (‰)	SD (‰)	Salinity
Mangrove leaves										
Rhizophora apiculata (L)[a]	Futabanahirugi	Feb. '97		13	0.17 ~ 1.21	4.356	0.150	−28.738	0.031	
R. apiculata (S)[b]	Futabanahirugi	Feb. '97		23	0.44 ~ 1.33	6.343	0.150	−26.705	0.016	
Brugiera sp.	Ohirugi zoku	Feb. '97		45	0.26 ~ 0.65	7.977	0.066	−27.040	0.023	
Particulate organic matter (POM)										
POM at 0 m	Suityuu kendakubutu	Nov. 18, '96	Stn. 8					−17.934	0.026	28.34
POM at 0 m	Suityuu kendakubutu	Nov. 18, '96	Stn. 7					−18.106	0.037	28.09
POM at 0 m	Suityuu kendakubutu	Nov. 18, '96	Stn. 1					−19.848	0.045	25.86
POM at 0 m	Suityuu kendakubutu	Nov. 18, '96	Stn. 2					−21.955	0.010	21.93
POM at 0 m	Suityuu kendakubutu	Nov. 18, '96	Stn. 3					−22.941	0.023	18.37
POM at 0 m	Suityuu kendakubutu	Nov. 18, '96	Stn. 4					−23.532	0.023	16.28
POM at 0 m	Suityuu kendakubutu	Nov. 18, '96	Stn. 5					−25.610	0.034	9.26

a): L; Large size. b): S; Small size.

gut contents were investigated in fish samples. Thereafter, each sample was dried at 60°C for about half to one day. At least 4 whole individuals of fish and shrimp were pooled and muscle tissues were carefully excised from each specimen with a clean scalpel, and only muscle tissue was used for isotope analysis. Particulate organic carbon (POC) samples were collected with precombusted (450°C) Whatman GF/F glass-fiber filters and dried at 60°C. In each analysis, dried samples were pretreated with concentrated hydrochloric acid (HCl) for removing carbonates since carbonates are known to interfere with C signatures. Dry animal tissues, particulate organic matter and leaf tissues were ground to a fine powder using a mortar and pestle, then subjected to combustion.

2) *Isotope analysis*

The organic materials were converted into CO_2 and N_2 gases by the sealed quartz tube combustion method[5]. CO_2 and N_2 gases were separated and purified cryogenically with liquid nitrogen and dry ice ethanol slush. Purified gases were introduced into an isotope ratio mass spectrometer (Finnigan Mat, MAT 252). A graphite reference material was used as a standard for carbon isotope ratio measurements. ^{13}C values are expressed relative to the VPDB (Vienna Peedee Belemnite) standard. High purity tank nitrogen gas was used as a working standard for the nitrogen isotope ratio measurements. This working standard was calibrated against N_1 and N_2 ammonium sulfate. ^{15}N values are reported relative to nitrogen in air. Stable carbon and nitrogen isotope ratios are represented by the following δ values:

$$\delta\ ^{13}C,\ \delta\ ^{15}N(‰) = [\{R_{sample}/R_{standard}\} - 1] \times 1{,}000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

Results and discussion

Carbon isotope ratio of POM showed a positive correlation with the level of salinity ($n = 7$, $r^2 = 0.960$) (Fig. 2). Carbon isotope ratio of POM (without stations 7 and 8 in Fig. 1) also showed a positive correlation with the total carbon content ($n = 5$, $r^2 = 0.914$) (Fig. 3), suggesting that an area with higher salinity contains a higher total carbon content with a higher POM δ ^{13}C. The content of phytoplankton carbon calculated from chlorophyll-a was almost stable (about 15–25% of total carbon), regardless of the POM δ ^{13}C level (Fig. 4). These

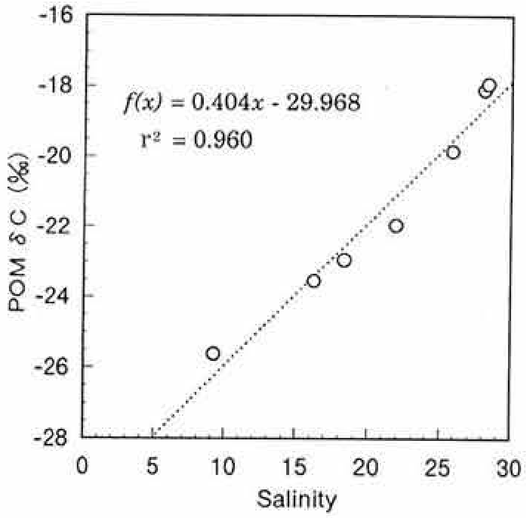

Fig. 2. Plot of carbon isotope ratio of particulate organic matter (POM) against the salinity at each sampling station in Sangga Besar, Matang

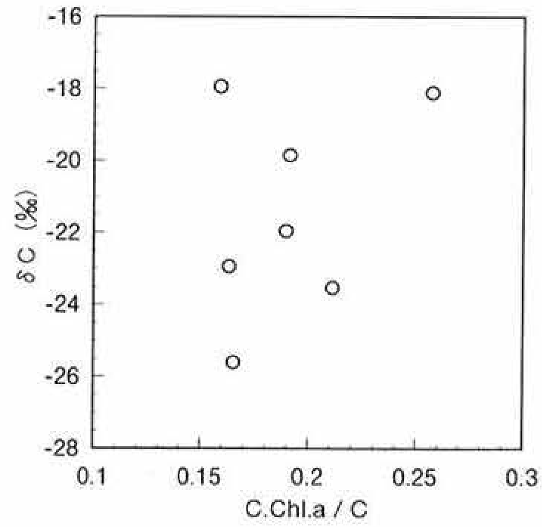

Fig. 4. Plot of carbon isotope ratio of POM against chlorophyll-a carbon content (total carbon basis) at the sampling station of Sangga Besar, Matang

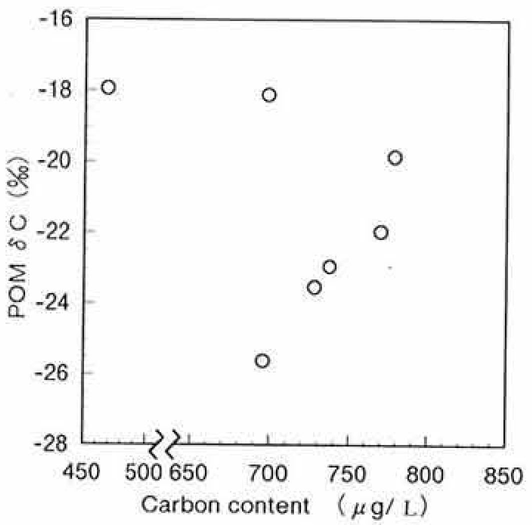

Fig. 3. Plot of carbon isotope ratio of POM against total carbon content at each sampling station in Sangga Besar, Matang

findings indicate that the phytoplankton-derived carbon contains about 15–25% of total carbon, and the major part of remaining carbon may consist of detritus carbon throughout the river to the estuary of Sungai Sangga. In other words, the results shown in Figs. 2–4 suggest that the POM around the mouth to mudflat areas of the river (higher POM $\delta\ ^{13}C$) may consist of phytoplankton-derived detritus from the sea, whereas POM from mid- to upstream areas of the river (lower POM $\delta\ ^{13}C$ areas) may consist of mangrove-derived detritus. $\delta\ ^{13}C$ and $\delta\ ^{15}N$ values of mangrove leaves were the lowest without a range of POM $\delta\ ^{13}C$ values in Sungai Sangga.

In Sungai Selinsing, squid showed the highest value of $\delta\ ^{13}C$ and tropical anchovy (*Stolephorus commersonii*) showed the highest value of $\delta\ ^{15}N$ (Table 1). $\delta\ ^{13}C-\delta\ ^{15}N$ map showed that there was a C–N isotope ratio gradient from the Matang mangrove brackish water ecosystems with the lowest value with mean (±SD) of $\delta\ ^{13}C$ (−28.74±0.031‰) and $\delta\ ^{15}N$ (4.36±0.15‰) for a mangrove leaf (*Rhizophora apiculata*), the highest value of $\delta\ ^{13}C$ (−20.45±0.026‰) for a squid (*Loligo duvaucelii*) and of $\delta\ ^{15}N$ (13.63±0.021‰) for a fish (*S. commersonii*). Except for one fish, *Anodontostoma chacunda*, all fishes showed higher $\delta\ ^{15}N$ values suggesting a higher trophic level. Penaeid shrimps (*Penaeus merguiensis, Metapenaeus brevicornis* and *M. ensis*) and mangrove crab (*Scylla serrata*) showed an intermediate C–N value between those of mangrove leaves and fish/squid. The range of the $\delta\ ^{13}C$ values for POM in Sungai Sangga overlapped the $\delta\ ^{13}C$ signal range for fishes, squids, shrimps and crabs in Sungai Selinsing (Fig. 5). Thus a clear C–N isotope ratio gradient in the Matang mangrove brackish water ecosystems could be detected.

Natural Environment Research Center[7] which conducted the isotope studies of mangrove leaves, land and aquatic animals, mangrove and marine mud sediments collected in the northern portion (around

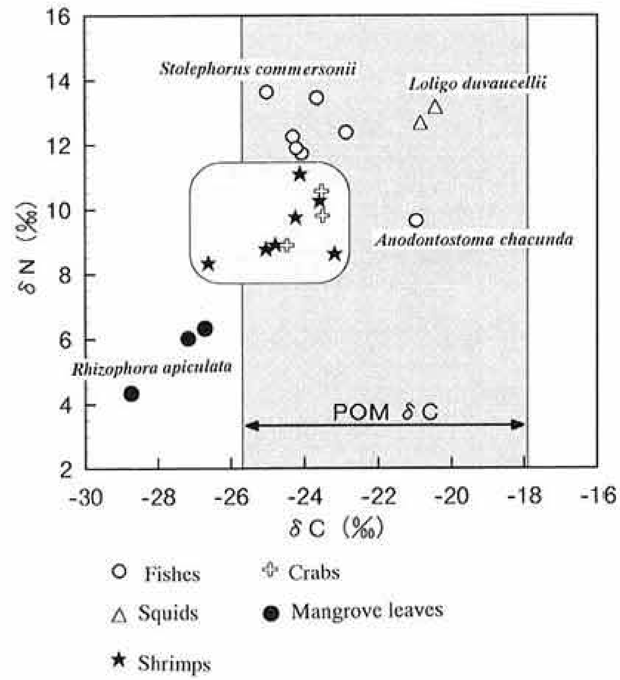

Fig. 5. $\delta\ ^{13}C-\delta\ ^{15}N$ map of mangrove leaves, animals and POM in the Matang mangrove brackish water ecosystem

Kuala Gula area) of the Matang mangrove ecosystem during the period 1992–1994, concluded the following: (1) there is a mangrove leaf-based organic matter flux in the water, running into the sea through the river, (2) some of the organic materials accumulated in the mud sediment and were utilized by mangrove/aquatic mollusks, and (3) fishes showed the highest values of $\delta\ ^{13}C$ and $\delta\ ^{15}N$, and also a clear C–N isotope ratio gradient from mangrove leaves up to fishes in the Matang mangrove brackish water ecosystems (Fig. 6).

The present results also suggest that there is a food pathway from the mangrove leaf-based detritus to carnivorous fishes and squids through herbivorous shrimps and crabs.

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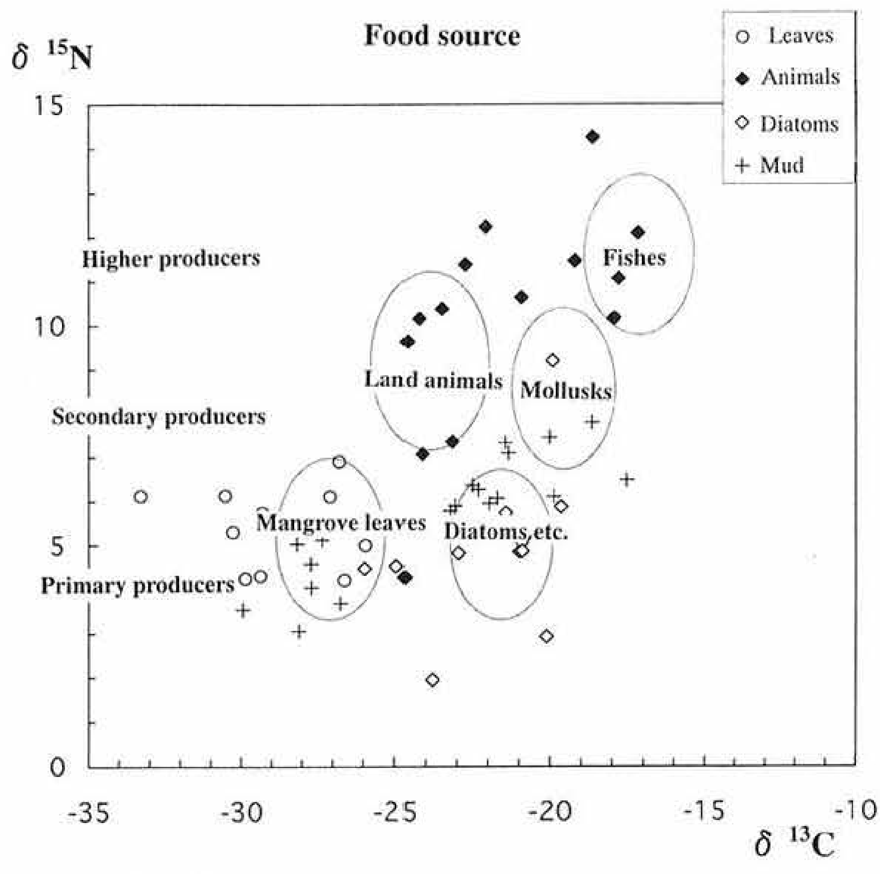

Fig. 6. $\delta\ ^{13}C-\delta\ ^{15}N$ plot and schematic map in the Matang (Sg. Gula) mangrove brackish water ecosystems (from Natural Environment Research Center[7])

(*Engraulis japonicus* larvae). Thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science (Agriculture) to the University of Tokyo, pp. 56.

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