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Stable isotopic structure of aquatic ecosystems

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Abstract. Isotopic, biogeochemical and ecological structure can provide a new dimension for understanding material flows, and the simultaneous function and structure of an ecosystem. Distributions of $\delta^{13}C$ and $\delta^{15}N$ for biogenic substances in the Nanakita river estuary involving Gamo lagoon in Japan were investigated to construct isotope biogeochemical and ecological structure for assessing fate and transfer of organic matter, and food web structure. The isotopic framework of the ecosystem was successfully described in a $\delta^{15}N$–$\delta^{13}C$ map. In this estuary the variations of isotope ratios of biogenic substances were clearly explained by the mixing of land-derived organic matter, and marine-derived organic matter.

A trophic-level effect of ^{15}N enrichment was clearly observed. Organisms were classified into three groups depending upon the contribution of land-derived organic matter in a food chain. Almost all biota except mollusca in the lagoon depend on organic matter of marine origin. The contributions of both land and marine organic matter were comparable for mollusca in the lagoon.

Keywords. Nitrogen isotope; carbon isotope; food web analysis; estuary; lagoon, watershed.

1. Introduction

Living organisms contain significant amounts of stable isotopes (SI) of light elements such as H, C, N, and O. Although the chemical nature of SIs is quite similar, each isotope has its own particular thermodynamic constant and rate constant These differ among SIs in all chemical and biological reactions. Variation in the isotope ratios of biogenic substances depends on the isotopic composition of reactant (substrate or diet), metabolic pathways, and kinetic modes of reaction dynamics. Every biogenic material thus has its own inherent isotopic composition, the so-called dynamic SI finger print (Wada and Hattori 1991). Consequently, the isotopic composition of an organism provides useful knowledge for diet analysis, such as the identity of nutrient sources and individual feeding behaviour, both of which determine an organism's function and position in the material flow of an ecosystem.

Nitrogen and carbon isotope ratios of phytoplankton vary spatially and temporally according to environmental conditions (Wada 1980; Takahashi *et al* 1990). The $\delta^{15}N$ is closely correlated with forms of nitrogen as well as organic growth rate (Wada 1980), and the $\delta^{13}C$ of plants is characterized according to CO_2

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assimilation systems known as 'C3'-plant and 'C4'-plant. The $\delta^{13}C$ becomes high in an aquatic environment where CO_2 diffusion is restricted (Sweeney *et al* 1978; Wada *et al* 1987).

The ^{15}N and ^{13}C contents of animals reflect their diets. Enrichment of ^{15}N through the trophic network is widely recognized among most animals, including invertebrates and vertebrates, leading to a value of 3·4 ± 1·1‰ (DeNiro and Epstein 1981; Minagawa and Wada 1984; Wada *et al* 1987). On the other hand, the $\delta^{13}C$ alues of animals vary, resembling those in their diet (Rau *et al* 1983; Fry *et al* 1984). These facts suggest that isotopic composition of aquatic organisms can provide basic information on their food source and trophic level. In theory, we can thus construct a SI food web ($\delta^{15}N$ *versus* $\delta^{13}C$) of an ecosystem. To realize this oal, isotopic ecological structure was determined in several aquatic ecosystems, including river estuaries and a lagoon and the Antarctic Ocean (Wada and Hattori 1991). We will here emphasize results obtained in Gamo lagoon nearby the Nanakita river estuary and the Otsuchi river watershed in Japan.

2. Materials and methods

Gamo lagoon is located in the estuary of the Nanakita river, Miyagi Prefecture, Japan (figure 1). It extends over 0·22 km^2 with 5 ha of tidal flats at low tide. Reed marshes have developed along the shore. Hydrographic observations of the lagoon were reported by Kikuchi *et al* (1980). Our second site, the Otsuchi river watershed, is located in the eastern part of Iwate Prefecture, Honshu, Japan. The upper reach of the watershed is mostly a mountainous area 750–800 m above sea level and the river starts 40 km from the opening of Otsuchi Bay. The lower reach of the watershed includes the town of Otsuchi and Otsuchi Bay. Further details of isotopic studies of this watershed are described elsewhere (Wada *et al* 1987). Samplings were performed at the lower reaches of the Nanakita river involving Gamo lagoon from 1982 through 1987.

Surface sediments were collected by using a shovel or a core sampler. All sediments were dried, crushed and homogenized. Particulate organic matter (POM) in surface-water samples were collected on a Whatman glass fiber filter (GF/C) by filtration. Plankton samples were obtained from surface water by horizontal tows of MTD nets. Benthic animals, sea shell and invertebrates were mostly collected in amo lagoon. Some fishes were collected at a small seagrass meadow (eelgrass, *Zostera marina* located at the mouth of Otsuchi river. After drying, the sample materials except fishes were ground, homogenized, passed through a 0·5 mm sieve and subjected to isotope analysis. For the fishes, only the muscle was used for the analysis.

Organic nitrogen (1–5 mg N equivalent) was converted to ammonia by Kjeldahl digestion. The ammonia thus produced was steam-distilled, concentrated, and then converted in *vacuo* to N_2 gas with alkaline hypobromite. The N_2 was purified by circulation through a CuO furnace with Pt wire (700°C) and a Cu furnace (400°C) for 30 min (Wada *et al* 1984).

After acidification with 0·5 N HCl, samples were freeze-dried and then converted to CO_2 by the combustion method, after mixing a sample with a mixed powder composed of oxidation catalysis (CuO:WO:VO= 100:1:1 in weigfit ratio). The

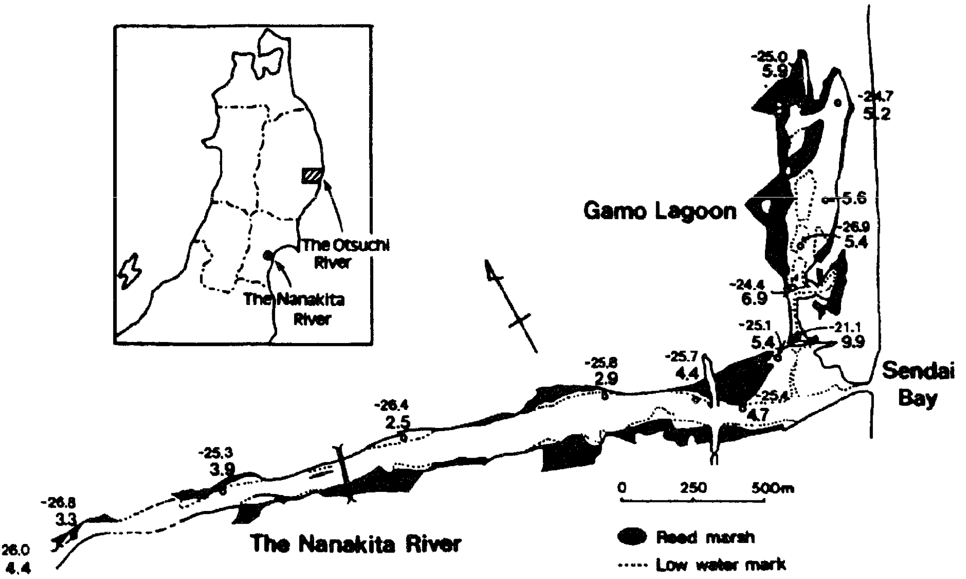

Figure 1. Sampling locations of the Nanakita river estuary and the Gamo lagoon (38°15'N, 141°01'E). Numerical value denotes δ^{13}C (top) and δ^{15}N (bottom) of sedimentary organics at indicated station.

evolved CO_2 was purified by the liquid nitrogen/dry ice-alcohol trapping method (Mizutani and Wada 1985).

Isotope ratios were measured using a Hitachi RMU-6R mass spectrometer fitted with a double collector for ratiometry, and expressed in per-mil deviation from a Standard as defined by the following equation:

$$\delta^{15}\mathrm{N} \quad \text{or} \quad \delta^{13}\mathrm{C}\,(‰) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000,$$

where $R = {}^{14}\mathrm{N}/{}^{15}\mathrm{N}$ or ${}^{13}\mathrm{C}/{}^{12}\mathrm{C}$, respectively. Atmospheric nitrogen (for δ^{15}N) and Peedee belemnite (PDB) (for δ^{13}C) were used as the standard. The standard deviations of isotopic measurements were less than 0·3‰.

3. Results

3.1 *The Gamo lagoon and the Nanakita estuary*

3.1a *Plant, POM and sediment*: Reeds collected from the marsh in the lagoon exhibited a δ^{13}C value of –26·8‰, while epiphytes gave –21·3‰. The δ^{15}N value of detritus originating from the reed was variable and extended from 3 to 7‰. The δ^{15}N of all plants ranged from 3 to 14·6‰. The average δ^{15}N of all plant materials in the lagoon was 8·6 ± 3·6‰ (average ± SD, $n = 7$). Sedimentary organics exhibited a rather constant δ^{13}C value (–26·4~ –24·4, average –23·5‰) throughout the river estuary and the lagoon. The corresponding δ^{15} N was low in the estuary (2·5

~3·9‰) and increased at the river mouth (4·4~5·4‰) to the lagoon (5·2~5·6‰) (tables 1 and 2).

3.1b *Benthic animals and macrofauna:* The δ^{13}C values of most of marine animals collected from the lagoon ranged from –22 to –20%, similar to the marine-derived organic matter (table 3). Several mollusca such as *Nuttallia olivacea, Corbicula japonica,* and *Laternula limicola,* however, gave rather high δ^{13} Cvalues of –24·2±0·6‰. The δ^{15}N value for the animals varied from 8·2 to 16·7‰. It increased in the following order: *Neanthes japonica* (8·3‰) in the river, Mollusca (9·4%o), *N. japonica* from the lagoon (11·8‰), Decapoda (12·7), pisces (14‰) and *Grandidierella japonica* (16·7‰).

Differences in the isotopic composition were observed among same species depending upon origin of the food source. For example *N. japonica* collected at the river estuary showed comparatively low isotopic compositions of δ^{15}N = 8·3‰ and δ^{13}C of –26·1‰, while the isotope structure was δ^{15}N=11·8 ‰ and δ^{13}C= –26·6‰ for the lagoon. *N. japonica* is well known as a surface-deposit feeder (Tsuchiya and Kurihara 1980).

The change in habitat seemed to affect the animal isotopic composition (table 4). The crab *Helice tridens* showed an approximately systematic variation in δ^{13}C with increasing carapace width to give the highest value of –18·5‰. Average δ^{13}C and δ^{15}N values of the present biogenic samples are summarized in table 5. The δ^{13}C value higher than –20‰ was found only for crabs with a carapace width greater than 2·5 cm. The *H. tridens* δ^{15}N value ranged from 11·2 to 14·0‰. The δ^{15}N value varied and increased with larger carapace width. A large variation in the δ^{15}N from 11·2 to 14·0‰ was found for *H. tridens* with a carapace width greater than 2·5 cm.

4. Discussion

Considerable data has accumulated on the distribution and variation of nitrogen and carbon isotopes. In general, nitrogen and carbon isotope fractionation take place at certain sites in an ecosystem, as indicated by the circled numbers in figure 2. In this diagram of an aquatic system, number 1 denotes occurrence of isotope fractionation that involves isotopic composition of substrates used for plant growth. Major substrates such as water, CO_2, HCO_3^-, and NH_3 undergo fractionation *via* abiological processes such as an exchange equilibrium reaction between CO_2 in the atmosphere and HCO_3^- in the sea, and the evaporation of seawater and NH_3. Redistribution of the isotopic compositions of these substrates by the physico-chemical processes provides a steady-state supply of the substrate with constant isotopic compositions in regional and/or global dimension (Wada 1986). Reactions 2 and 3 contribute to isotope fractionation between substrate and primary producers. The isotopic compositions of substrate, the reversibility of substrate transport through (reaction 2) the supply of reducing substrate (for example light intensity), and the inherent isotope fractionation of enzyme-substrate determine the magnitude of isotope fractionation between substrate and primary producers. In general, the fractionation becomes large when substrate concentrations do not limit the reaction rate and ^{15}N and ^{13}C content in primary producers is lower than that

Table 1. Summary of $\delta^{13}C$ and $\delta^{15}N$ values of sedimentary organics from the Nanakita river estuary and Gamo lagoon.

Sample	Sampling time	Carbon dry weight (%)	Nitrogen dry weight (%)	C/N	$\delta^{13}C_{PDB}$ (‰)	$\delta^{15}N_{air}$ (‰)	Remarks
River sediment*	Summer, 1982	2·34 ± 0·45	0·162 ± 0·027	14·4 ± 1·8	−25·8 ± 0·5	4·3 ± 1·0	Mid-river $n = 5$
	Summer, 1983	1·49 ± 0·32	0·121 ± 0·031	12·4 ± 0·9	−25·6 ± 0·3	4·2 ± 0·4	Tidal flat $n = 12$
Lagoon sediment	Summer, 1982	0·27	0·037	7·3	−26·9	5·8	0–2 mm
		3·8	0·28	13·6	−26·5	5·9	10 cm depth
	Summer, 1983	4·47	1·19	3·8	−25·6	7·5	Surface 0·1 mm
		2·24	0·22	10·2	−26·2	6·8	Reed marsh 0·1 mm

*Mean ± SD (number of samples). Sediment samples collected in summer, 1986 are indicated in figure 1.

Table 2. Summary of $\delta^{13}C$ and $\delta^{15}N$ values of plant and suspended organic matters collected from Gamo lagoon.

Sample	Sampling time	Carbon dry weight (%)	Nitrogen dry weight (%)	C/N	$\delta^{13}C_{PDB}$ (‰)	$\delta^{15}N_{air}$ (‰)	Remarks
Lagoon							
Epiphyte	Summer, 1987	10·3	1·4	7·4	−21·3	14·6	1
Enteromorpha		23·4	3·1	7·5	−20·6	10·0	2
Sargassum		23·5	1·5	15·7	−19·1	9·2	3
Reed marsh	Summer, 1982						
Reed		42·7	4·1	10·4	−26·8	7·0	4
Reed remain		40·1	3·5	11·5	−24·8	6·9	5
Reed detritus		36·4	2·6	14·0	−26·9	3·0	6
Lagoon	Aug., 1983						
Flooting mud		2·73	0·36	7·6	−23·7	9·3	7
		4·94	0·77	6·4	−23·5	10·2	8
		2·32	0·53	4·4	−23·4	9·7	9
Mud from the inner part	Summer, 1984	ND	0·603	ND	ND	5·4	10
Microcysis spp. in the farm pond	Summer, 1984	ND	7·68	ND	ND	9·5	11 Farm pond

ND. Not determined.

Table 3. Summary of $\delta^{13}C$ and $\delta^{15}N$ values of animals collected from Gamo lagoon.

Sample	Sampling time	Carbon dry weight (%)	Nitrogen dry weight (%)	C/N	$\delta^{13}C_{PDB}$ (‰)	$\delta^{15}N_{air}$ (‰)	Remarks
Amphipoda							
Grandidierella japonica	Aug., 1983	19·8	5·9	3·4	−21·2	16·7	12
Lilljeborgia japonica	Aug., 1984	31·7	7·2	4·4	−20·3	10·2	13
Polychaeta							
Neanthes japonica	July, 1983	50·0	12·0	4·2	−22·1	11·8	14 Large size
					−21·0	11·8	15 Small size
					−26·5*	8·2*	16 Large size
					−25·7*	8·4*	17 Small size
Mediomastus californiensis					−21·2	14·2	18
Prionospio japonicus					−21·7	ND	19
Mollusca	July, 1983						
Assiminea lutea japonica		26·6	7·0	3·8	−21·5	10·1	20
Nuttalia olivacea		40·2	6·5	6·2	−24·2	9·1	21
Corbicula japonica		ND	ND	ND	−24·5	9·9	22
Laternula limicola		ND	ND	ND	−23·5	8·5	23
Decapoda (Brachyura)	July, 1983						
Hemigrapsus penicillatus		42·2	7·5	5·6	−22·2	12·9	24
		45·5	6·7	6·8	−22·8	12·8	25
Pisces							
Limanda yokohamae		39·7	10·6	3·7	−21·0	13·4	26
Acanthogobius flavimanus		31·2	10·6	2·9	−21·2	14·6	27

ND, Not determined.
*Samples were collected from the Nanakita river.

Table 4. Isotopic compositions of *Helice tridens.*

Sample No.	Carapath width (cm)	Carbon dry weight (%)	Nitrogen dry weight (%)	$\delta^{13}C_{PDB}$ (‰)	$\delta^{15}N_{air}$ (‰)
Summer, 1983					
1	1·0–1·5	38·5	ND	−22·1	12·4
2	1·5–2·0	ND	ND	−21·7	12·8
3	2·0–2·5	ND	ND	−22·1	12·4
4	2·5–3·0	ND	ND	−20·6	12·0
5	3·0–3·5	ND	ND	−18·5	11·9
June 2, 1984					
7	1·69	35·3	14·8	−21·8	12·7
8	1·71	34·7	13·5	−22·1	11·7
9	1·85	37·2	12·6	−21·2	11·8
10	2·34	36·7	13·3	−22·0	13·1
11	2·51	36·7	12·9	−21·1	13·6
12	2·74	31·5	13·2	−19·8	14·0
13	2·87	33·9	12·5	−22·1	11·5
14	2·92	35·0	12·3	−21·8	11·2
15	3·18	36·2	12·7	−18·8	13·8
16	3·29	35·0	12·1	−18·9	12·6

ND,Not determined

Table 5. Carbon and nitrogen isotope ratios for various samples in the Nanakita river estuary together with the Gamo lagoon.

Sample	$\delta^{13}C_{PDB}$ (‰)	$\delta^{15}N_{air}$ (‰)
Nanakita river		
River sediment	−25·8 ± 0·5 (6)	4·3 ± 0·9 (6)
Lagoon sediment	−25·7 ± 0·5 (11)	4·7 ± 0·9 (3)
Sediment in reed marsh	−25·8	3·9
Gamo lagoon		
Floating mud	−23·5 ± 0·2 (3)	9·7 ± 0·5 (3)
Animal	−22·0 ± 1·3 (14)	12·0 ± 0·5 (3)
Reed	−25·8 ± 1·4 (2)	7·0 ± 0·1 (2)
Sediment in reed marsh	−26·2 ± 0·3 (3)	6·7 ± 0·8 (3)

Mean ± SD (number of samples)

under substrate-limiting conditions. The number 4 denotes the occurrence at a branch point in metabolic pathway. Lower δ^{13}C values of lipid fractions in almost all organisms than those of corresponding whole-body and other protein-rich fractions, are ubiquitously recognized (Monson and Hayes 1980). Reaction ·5 signifies ^{15}N enrichment within a single feeding process (Minagawa and Wada 1984). The enrichment has a value of 3·4±1·1‰ irrespective of biochemical differences, such as the existence of a urea cycle, the production of uric acid, or differences in food habitat (figure 3a). A clear-cut relationship between animal δ^{15} N and its trophic level (TL) was found in the Antarctic Oceanic ecosystem as follows:

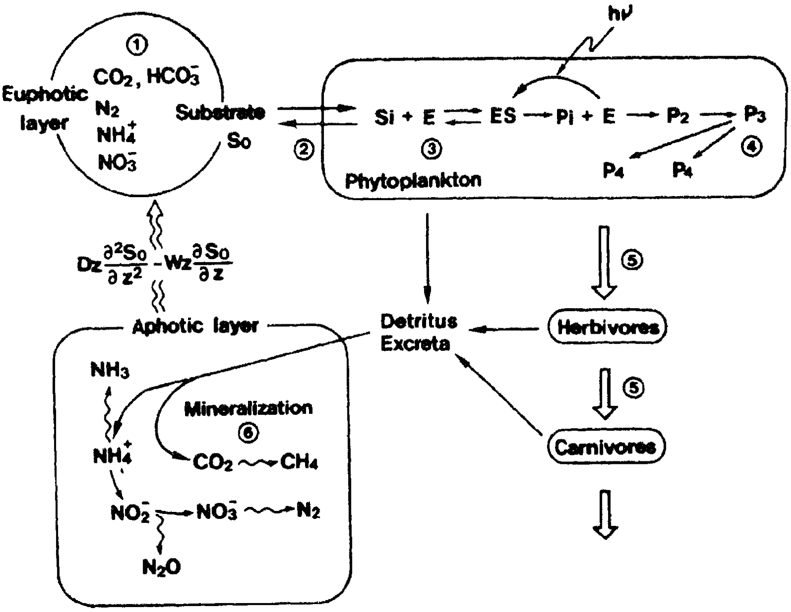

Figure 2. Schematic model for carbon and nitrogen isotope fractionation in an aquatic ecosystem. The circled numbers denote major sites of isotopic variations. (1) Isotopic fractionation during biogeochemical matter cycling; (2, 3) kinetic isotope effects in the uptake processes by plants; (4) branch point in metabolic pathway; (5) enrichment of ^{15}N in a feeding process; and (6) mineralization to produce gaseous products. (From Wada and Hattori 1991).

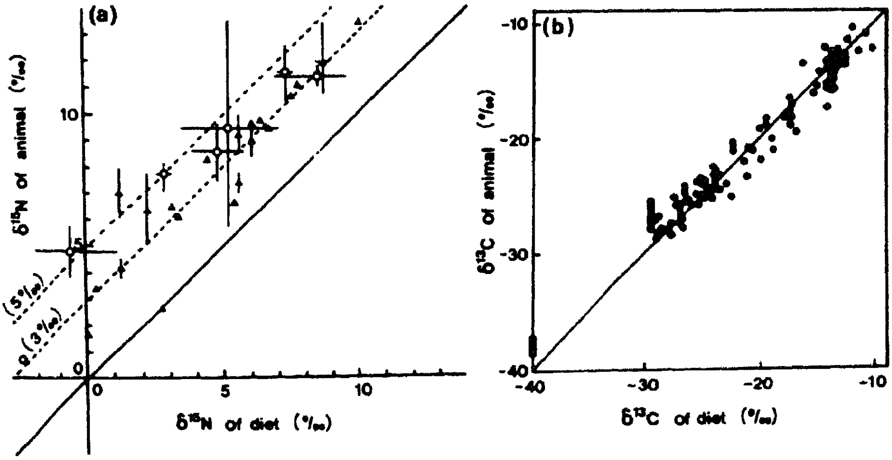

Figure 3. **(a)** ^{15}N in animal bodies as a function of ^{15}N in diet. Open circles mean data from field observations. Solid triangles are those from culture experiments These data include vertebrates and invertebrates (figure was made from data given by Minagawa and Wada 1984). **(b)** Relation between animal $\delta^{13}C$ and diet $\delta^{13}C$. Solid line means no isotope fractionation. (From Fry and Sherr 1984).

$$\delta^{15}N\ (animal)‰ = 3{\cdot}3\ (TL-l) - 0{\cdot}2\ (\delta^{15}N\ of\ diatom),$$

where TL=1 denotes TL of diatom (Wada *et al* 1987). The enrichment factor of 3·3 will be applied for diet analysis in the present fields. On the other hand, the δ^{13}C values of animal tissue are very close to those in their diet, and a small increase in ^{13}C content (less than 1‰) sometimes occurs in a predator as compared to its diet (Fry and Sherr 1984) (figure 3b). It is well-known that isotope fractionation factors are large, up to 1·05 during the mineralization of organic matter to gaseous compounds such as N_2O. N_2 and CH_4 (Wada and Hattori 1991; Whiticar *et al* 1986). Low δ^{15} N values were found for POM (0·2 to 0·7‰) in the upper reaches of the Otsuchi river watershed, while high δ^{15}N values of 6·4±1·8‰ were found in the Otsuchi Bay (Wada *et al* 1987). Similar differences in δ^{13}C value (4·5‰) were found for POM between the upper reaches (–26·0±0·5‰) and the Bay (–21·5±0·5‰). The nitrogen and carbon isotope ratios found in sedimentary organic matter in the Otsuchi Bay were explained by the mixing of land- and marine-derived organic matter (Wada *et al* 1987). Since both the Otsuchi watershed and the Nanakita river are located in the same district, the northern part of Honshu Island, Japan, the relation can also be applied to the latter area for assessing the contribution of land-derived organic matter to living organisms as well as sedimentary organics. There are several seagrass meadows located at the Otsuchi river mouth. Eelgrass (Z. *marina)* shows high δ^{13}C values of – 9 to – 14‰ and δ^{15}N of about 7‰ similar to those of phytoplankton in the Bay. This community supports an active habitat for macrofauna as well. In fact, fishes collected from the seagrass meadow exhibit δ^{13}C of –18 to –14‰ and δ^{15}N of 9 to 15‰, respectively (figure 4, Gl-6). Keeping these facts in mind, the present isotopic data were examined for assessing the SI structure of the estuarine ecosystems including the lagoon in Japan.

In sedimentary organic matter and living organisms in the two estuaries, δ^{13}C and δ^{15}N were noted to increase from the upper reaches to the intertidal zone, and then to the bay mouth area. Essentially the same has been found for estuarine areas of Tokyo Bay (Wada *et al* 1990). Wada *et al* (1987) explained this trend as follows: (i) mixing of terrestrial and marine organic matter, (ii) rapid growth of phytoplankton on nutrient salts transported via rivers in an estuarine zone, and (iii) discharge of sewage and waste water with high δ^{15}N from surrounding cities. In the case of the Gamo lagoon, a farm pond adjacent to the lagoon discharged sewage as indicated by a high δ^{15}N value (9·5‰) of *Microcystis* spp. (table 2).

The isotope biogeochemical-ecological structure of the Otsuchi river estuaries is schematically presented in figure 4. The structure was constructed principally using the three kinds of primary producers (land plant, marine algae, and seagrasses) with different isotopic compositions (Wada *et al* 1987) and food chain effects, as indicated by T-, M-, and G-types of food chain.

Here a mixing curve (a line T_1M_1 in figure 4) of the former two sources is adopted from the results obtained in a Otsuchi watershed. Macrofauna collected from the present estuarine systems were divided into three groups as indicated by a land-type (D), a marine-type (F) and a seagrass-type (H). However, several kinds of fishes collected from the seagrass meadow (Gl-6) are placed between last two types as indicated in figure 4. The flow of organic matter to these fishes had originated from phytoplankton and seagrasses, simultaneously.

In conclusion, the variation of isotope ratios in the estuaries was explained by the

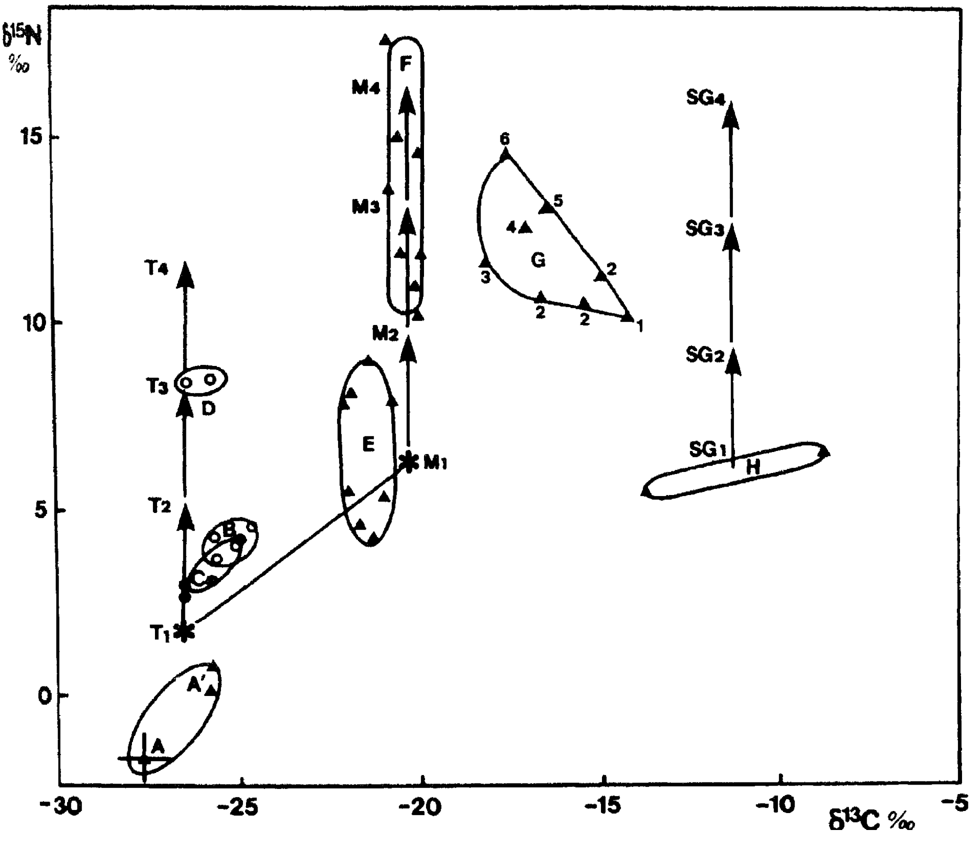

Figure 4. δ_{15} N – δ_{13} C map of varioust biogenic substances from the Otsuchi watershed system.Line TM denotes the mixing line obtained from the Otsuchi Bay sediment. (A) Plant remains in the upper reaches, (A') POM in the upper reaches, (B) mountain soil, (C) river sediments in the upper reaches, (D) benthic animals in the river, (E) POM in the bay, (F) netted plankton in the bay, (G) fish collected from the eelgrass meadow in July 1981, Gl, *Enedrias nebulosus:* G2, *Ditrema temmincki;* G3, *Syngnathus schlegeli;* G4, *Tribolodon hakonensis;* G5, *Pseudoblennius cottoides;* and G6, *Sebastes inermis,* and (H) seagrasses at the inner bay. Numerical suffixes stand for the trophic level in each ecosystem. (From Wada E 1987 *Isotopen praxis* **23** 320 with permission.)

mixing of land-derived organic matter, marine phytoplankton, and seagrasses. Consequently, a rather simple isotopic structure was found for complicated stuarine systems in the δ^{15}N – δ^{13}C map as indicated in figure 4.

The percentage contribution of land-derived organic matter to total sedimentary organics (f_c) was calculated for the lagoon sediments using the equation given by Wada *et al* (1987).

$$f_c\% = \frac{\delta^{13}\mathrm{C}_{\mathrm{marine}} - \delta^{13}\mathrm{C}_{\mathrm{sediment}}}{\delta^{13}\mathrm{C}_{\mathrm{marine}} - \delta^{13}\mathrm{C}_{\mathrm{land}}} \times 100$$

$$= \frac{-20{\cdot}3 - \delta^{13}\mathrm{C}_{\mathrm{sediment}}}{6{\cdot}2} \times 100.$$

As indicated in table 5, the average δ^{13}C values of the river, lagoon and reed mash sediments was – 25·8 ± 0·5‰, accounting for *ca.* 90% of f_c, that is around 90% of organic matter in the estuarine sediment was derived from the Nanakita river. On the other hand, floating mud collected in Gamo lagoon gave the δ^{13}C value of -23·5‰ as an average. The f_c value for this material was approximately 50%, and therefore less from the river and more from the marine environment.

The δ^{15}N and δ^{13}C values of the lagoon animals are plotted in more detail in figure 5. Almost all animal δ^{13}C values varied between – 22 and –20‰ suggesting

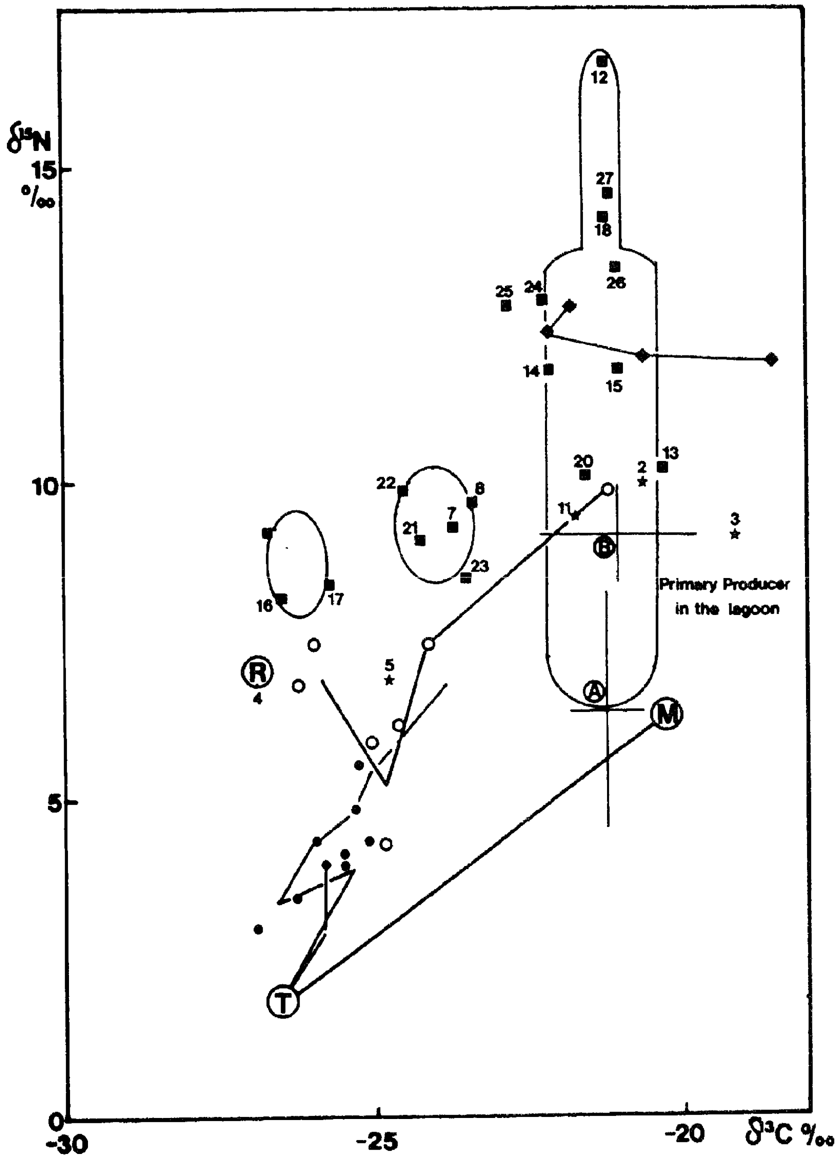

Figure 5. δ^{15}N–δ^{13}C map of various organisms from the Gamo lagoon and Nanakita river estuary. Line TM, see figure 4; (O), sedimentary organics in the lagoon; (●), sedimentary organics in the river estuary; (R), reed; and (♦), *H. tridens*. Numerical values denote organisms appeared in tables 2 and 3 (see remarks). (A) particulate organic matter in Otsuch Bay, and (B) primary producers in the lagoon.

that the organisms in this food web largely originated from marine primary producion. Exceptions were three kinds of mollusca (21, 22, 23) (δ^{13}C = – 24·2 ± 0·6‰) and the contribution percentage (f_{c}.) accounted for around 50% for these organisms, like that of the floating mud (7, 8 in figure 5). These mollusca were reported to be suspension or surface-deposit feeders (Tsuchiya and Kurihara 1980; E Kikuchi, personal communication). Three primary organic sources (T, R, and B in figure 5) are considered in the Gamo lagoon. These are the land-derived organic matter (T), phytoplankton (B) and the reeds (R) in the salt marsh. Judging from the high δ^{15}N values of the reed, its surrounding detritus and *Microcystis* spp. in the farm pond, oxidation-reduction processes operating with gaseous production (reaction 6 in figure 2) seemed to prevail in the salt marsh areas. An increase in δ^{15} N among macrofauna can be explained by the enrichment of ^{15}N along the food chain if we take food habitat of each animal into consideration. For example *G. japnoica* gave the highest δ^{15}N value of 16·7‰ . The organism has potential ability to feed on mud organic matter and dead fish with high δ^{15}N (E Kikuchi, personal communication). In conclusion, we can find three kinds of food chain in the Gamo lagoon. Food bases of these groups are the land-derived organic matter, floating mud, and marine phytoplankton (figure 5).

Small individuals of *H. tridens* burrow in the reed marsh and feed on detritus in the marsh at ebb tide, while large individuals exhibit exploratory behaviour for feeding. The large ones migrate into the lagoon outside the marsh and feed on a wide variety of food such as the dead bodies of fish, seaweed and detritus (Takeda et al 1988; Kurihara *et al* 1988, 1989). The food habit of *H. tridens* by δ^{15} N and δ^{13}Canalyses is clearly indicated in figure 6. Variation in δ^{15}N for large *H. tridens* individuals ranged from 11 to 14‰, strongly suggesting that their food sources become more widely varied as their home range widens. Food sources seemed to be diverse, judging from the variation of the δ^{15}N around 3‰ (corresponding to one trophic level). On the other hand, the higher δ^{13}C value of more than –20‰ was found for the large *H. Tridens* individuals, indicating that almost all their food was derived from marine organic matter, while small *H. tridens* individuals fed on a mixture of land- and marine-derived organic matter.

Ecological and biogeochemical processes of an aquatic ecosystem are schematically summarized in figure 7, described by physical and chemical parameters. In the marine environment, primary production by phytoplankton and decomposition of organic matter are represented with a Standard stoichiometric,. RKR model as shown in figure 7 (Wada and Hattori 1991). Contribution of land-derived organic matter to sedimentary organics (*f*) can be calculated by isotope mass balance: $\bar{\delta}$(mean)=$\Sigma f_{i}\delta_{i}$. The enrichment of ^{15}N during single feeding process provides the relation between an animal's trophic level and its δ^{15}N as:

$$\mathrm{TL}=\frac{1}{3\cdot3}\left(\delta^{15}\mathrm{N}_{\mathrm{animal}}-\delta^{15}\mathrm{N}_{\mathrm{phytoplankton}}\right)+1.$$

In the present estuarine system, the enrichment factor of 5 ± 2 was obtained for *N. japonica* as indicated in figure 7. This figure can thus provide a new paradigm for assessing food-web structure and its perturbation, and matter cycling in an aquatic ecosystem. Some examples can be emphasized. Eelgrass meadows provide ^{13}C enriched ecosystems, of which δ^{13}C values (– 18 – – 14‰) was highly different from those of phytoplankton in the surrounding seawater (figure 4). The δ^{13}C variation

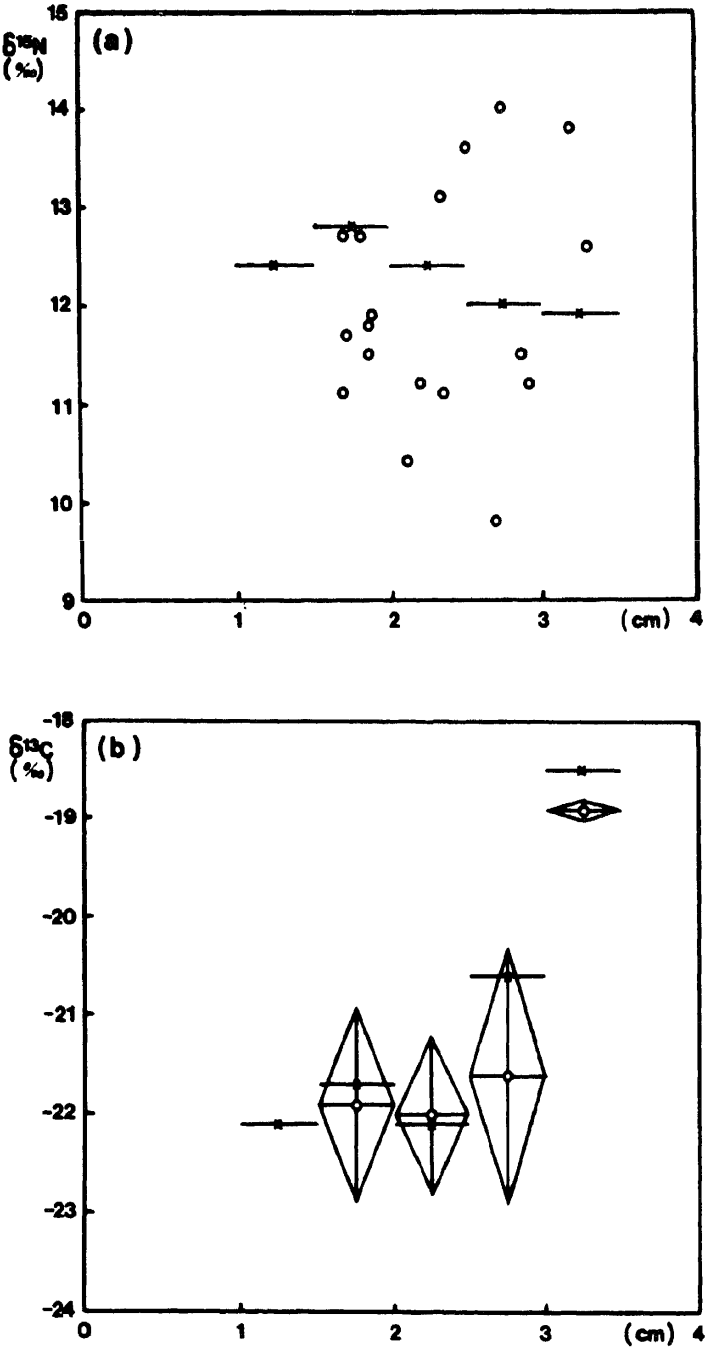

Figure 6. **(a)** Relation between the $\delta^{15}N$ of *H. tridens* and each corresponding carapace width, (x) Samples were collected in 1983. (O) Samples were collected on 2 June, 1984. **(b)** Relation between the δ1 3 C of *H, tridens* and each corresponding width.

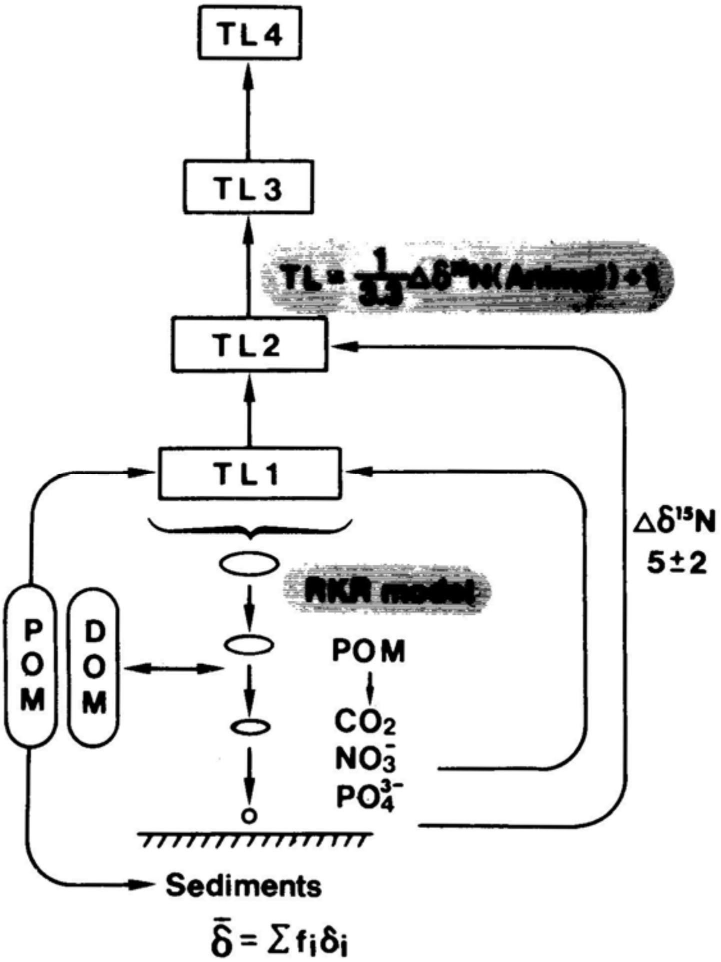

Figure 7. Biogeochemical framework of an aquatic ecosystem. RKR (A C Redfield, B H Ketchum and F A Richards) model means the decomposition of organic matter in seawaters: $(CH_2O)_{106}(NH_3)_{16}PO_4 + 138\ O_2 \rightarrow 106\ CO_2 + 16\ HNO_3 + H_3PO_4 + 122\ H_2O$.

among fish collected from the eelgrass meadow indicated that the contribution on he seagrass to the total food base of each species was different as indicated in G1 to G6 in figure 4.

Differences in isotopic compositions were also observed for *N. japonica* (Nos. 14 to 17 in figure 5). The animal isotopic compositions varied in association with origin of food source: land-derived or marine organic matter.

The temporal variation of patterns of δ^{15}N between the mysid and its food sources (particulate organic matter) was parallel in Lake Kasumigaura, Japan (Toda and Wada 1990). The (δ^{13}C and δ^{15}N values of *Hypomesus* (a pond smelt), which is at the highest trophic level in Lake Suwa, Japan also reflected the temporal changes in those of phytoplankton (Yoshioka 1991).

Human impact such as dam construction and deforestation enhanced the release of biogenic gases in tropical aquatic ecosystems in Brazil (Wada *et al* 1991). Consequently, δ_{15}N of remaining available nitrogen became higher and high δ^{15}N values for aquatic algae were recorded.

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