Carbon and nitrogen stable isotope ratios of deposit-feeding polychaetes in the Nanakita River Estuary, Japan

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Abstract

Two types of deposit-feeding polychaetes, *Neanthes japonica* and *Notomastus* sp., and their surrounding sediments were collected from the Nanakita River Estuary and a small brackish lagoon (Gamō Lagoon) in northeastern Japan. The samples were examined using stable isotope analysis to assess the site specific feeding mode of the animals and their trophic status. *N. japonica* is a surface deposit-feeder and *Notomastus* sp. is a subsurface deposit-feeder. In the estuary, the sedimentary $\delta^{15}N$ tended to become isotopically heavier from the upper estuary (2.0 ∼ 3.9‰) to the river mouth (4.3 ∼ 6.2‰), while sedimentary organic $\delta^{13}C$ exhibited no such trend and maintained a rather constant value (−26.8 ∼ −24.4, average -25.6‰) throughout the river estuary. The $\delta^{13}C$ values of *N. japonica* were similar to those of the surrounding sediment in the upper estuary, whereas in the lower estuary, *N. japonica* had a heavier $\delta^{13}C$ value than the surrounding sediment. The $\delta^{13}C$ and $\delta^{15}N$ values indicated that the carbon, but not the nitrogen, of *N. japonica* was derived from upland plants in the upper estuary. In the lower estuary, a significant fraction of carbon of *N. japonica* was derived from phytoplankton. *Notomastus* sp. exhibited heavier $\delta^{13}C$ values than the surrounding surface sediment throughout the estuary and had heavier $\delta^{13}C$ values than *N. japonica* in the same location. These results suggest selective utilization of sedimentary carbon by those animals following bacterial processing and subsequent fractionation. The difference in $\delta^{15}N$ between sedimentary organics and corresponding polychaetes was 5 ± 1‰ and rather higher than 3.4 ± 1.1‰ expected for normal trophic effects in other animals.

Introduction

Stable isotope analysis is now widely used as a means of tracing material flow through food chains (see Fry & Sherr, 1984, for a review). The stable isotope ratio of organic carbon has been found to be an excellent tracer to identify the diet of animals, since only minor changes in the carbon isotope ratio occur with each trophic transfer (DeNiro & Epstein, 1978; Frey *et al.*, 1978; Thayer *et al.*, 1978; Fry & Sherr, 1984). On the other hand, ^{15}N is more considerably enriched (3.4 ± 1.1‰) in consumers than in their food (Rau, 1981; Minagawa & Wada, 1984). Because the difference in $\delta^{15}N$ values is significant even in a single trophic transfer, the nitrogen isotope ratio can be used as a tracer not only for dietary analysis, but also for

determining the trophic level of given animals (Minagawa & Wada, 1984; Wada *et al.*, 1987a).

Many studies have documented the value of the isotope method in identifying food sources for animals that live in estuarine environments where the diet is derived from several sources such as rivers, marine sources, and peripheral salt marshes, as well as from autochthonous material (Haines & Montague, 1979; Simenstad & Wissmar, 1985; Peterson & Howarth, 1987; Wada *et al.*, 1993). However, the dominance of the detritus pathway of material flow in estuaries may make the isotope analysis of the food webs very difficult, because the isotope composition of sedimentary organic matter gradually changes during degradation and biochemical processing by microbes (Owens & Law, 1989). In addition, the isotopic composition of

estuarine consumers is affected by their feeding modes as well as their location (Peterson *et al.*, 1985; Peterson & Howarth, 1987).

Deposit-feeding infauna are thought to form important links between sedimentary organic matter and carnivorous fish in estuaries. Estuary sediment serves as a food source for deposit-feeding invertebrates, but the main source of food for these animals has not been fully elucidated. Two genera of deposit-feeding polychaetes, *Neanthes japonica* (Izuka) and *Notomastus* sp., occur commonly and together in brackish intertidal and shallow subtidal sediments in Japanese estuaries. They are found in sediments from the upper estuary to near the mouth of estuary, since they both tolerate a wide range of salinity. Furthermore, *N. japonica* is a surface deposit-feeding nereid polychaete and *Notomastus* sp. is a deep deposit-feeding capitellid polychaete. In this paper, we present $\delta\ ^{13}C$ and $\delta\ ^{15}N$ data of these two deposit-feeding polychaetes and their surrounding sediments in the Nanakita River Estuary and compare them at different locations from the upper estuary to the mouth of the estuary to assess site specific feeding modes and isotopic changes with trophic transfer.

Materials and methods

Study area

The study was conducted in the Nanakita River Estuary in northeastern Japan (38°C 15'N:141° 1'E, Fig. 1), where the Nanakita River opens into Sendai Bay facing the Pacific Ocean. The Nanakita River is about 45 km long with a drainage area of c. 200 km². There is a weir about 4.2 km upstream of the mouth and the reaches downstream of the weir are regarded as the usual estuarine part. The tidal range in the Nanakita River Estuary is about 80 cm at spring tide, which is generally about half that of Sendai Bay, and the tidal phase lags c. 2–3 hours due to the shallow and narrow mouth. As a whole, this estuary has the salinity distribution of a salt-wedge type (Hanawa & Sugimoto, 1979). During flood tide, sea water flows into the estuary and intrudes up to 500 m upriver through the shallow narrow mouth. Then some portion of the water intruding into the estuary returns back to the sea during the ebb phase, while some water is entrained. The residual part penetrates upstream and circulates in the salt wedge (Hanawa & Sugimoto, 1979). Salinity in the salt wedge is about 25‰.

A sack-shaped lagoon (Gamō Lagoon) occurs on the north side of the river mouth c. 0.25 km upstream of Sendai Bay (Fig. 1). The lagoon (total area ≈ 10 ha; volume ≈ 1 to 5 × 10^4 m³) is separated from Sendai Bay by a flat shallow sand bar c. 200 m wide. A stone levee with two concrete pipes about 0.7 m in diameters forms the lagoon gateway through which its water is exchanged during tidal movement. The water depth at the deepest part of the lagoon is about 30 cm at low water and the tidal range is about 50 cm. The water column of the lagoon is well mixed and the salinity ranges from 2 to 30‰. A 12 ha carp pond located on the west side occasionally releases effluent into the lagoon and supplies the lagoon with suspended solids in the summer. There are narrow salt marshes dominated by *Phragmytes communis* (above ground part, $\delta\ ^{13}C$ – 26.8‰, $\delta\ ^{15}N$ 7.0‰; Wada *et al.*, 1993) on the shores of the Nanakita River and Gamō Lagoon.

Sampling

Sediment and polychaete samples were collected from intertidal flats of the Nanakita River Estuary including the lagoon part (Fig. 1) from September to October. *N. japonica* and *Notomastus* sp. were found to be the most abundant polychaetes in the Nanakita River Estuary. *Notomastus* sp. is a motile deposit-feeder which feeds on black anoxic mud at depths and egests ellipsoidal fecal pellets onto the sediment surface. It builds subvertical, partially irregular-shaped burrows, which maintain contact with the surface and extend to 20 cm below the surface (Kikuchi, 1987). The burrows are temporary structures without distinct oxidized brown linings and collapse within a few days after abandonment. *N. japonica* is a deposit-feeder which feeds on surface sediment. The semi-permanent U-formed burrow is vigorously irrigated by the peristaltic movement of the worm and an oxidized brown layer is observed around the burrow (Kikuchi, 1987).

Samples of live estuarine polychaetes collected *in situ* were washed free of contaminating mud and debris. They were held alive in diluted seawater in the laboratory for 4 to 8 h to clear their guts and then dried in a 60 °C oven. Animal tissue (more than 10 individuals) was pooled and ground by a mortar and pestle after drying. Sediment samples were collected by scraping the upper 1 cm of the exposed tidal flat surface. All sediments were treated with 0.5 N HCl, washed with distilled water, dried in a 60 °C oven, ground, homogenized, and subjected to isotope analysis. Therefore, $\delta\ ^{13}C$ and $\delta\ ^{15}N$ were measured for total
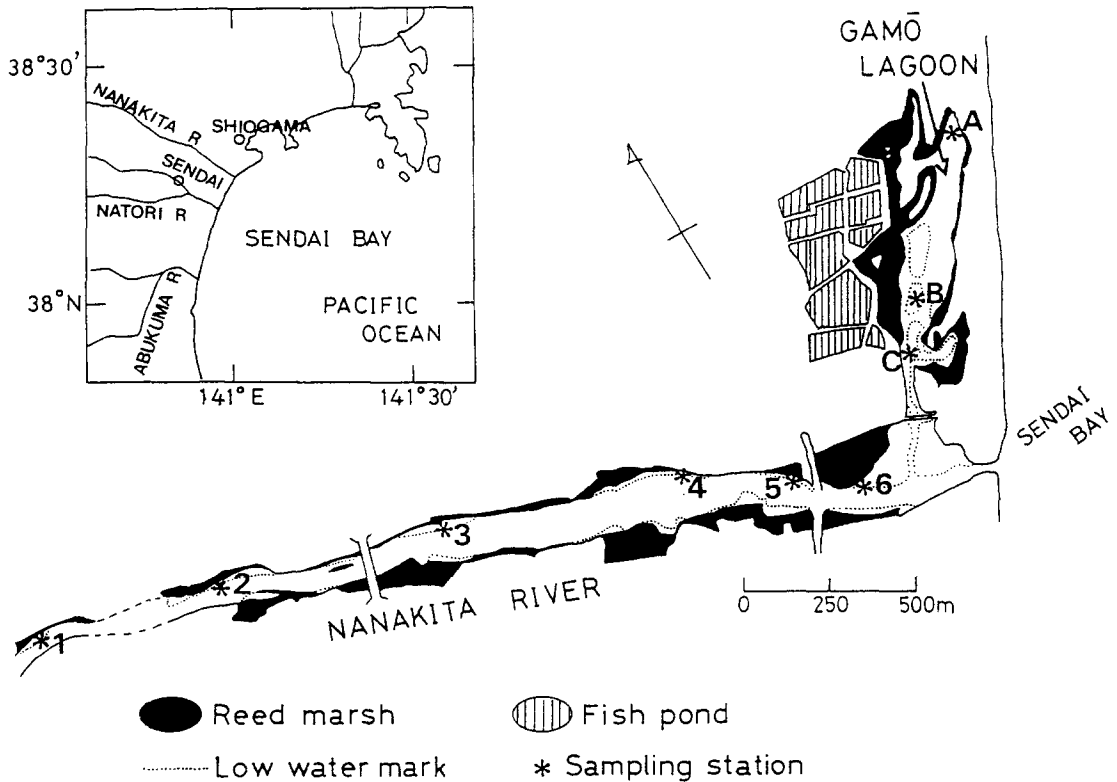
Fig. 1. Sampling locations in the Nanakita River Estuary (Sts. 1–6) and Gamō Lagoon (Sts. A–C).

organic carbon and total nitrogen including exchangeable ammonium in this experiment. Thus in this study, $\delta^{15}N$ of the polychaetes is compared with that of sedimentary total nitrogen instead of total organic nitrogen, because exchangeable ammonium accounts for only a small fraction of the sedimentary total nitrogen of the tidal flats in the Nanakita River Estuary (Sayama & Kurihara, 1983).

Isotopic analysis

The dried samples were treated to yield pure CO_2 and N_2 gas for isotopic determinations following the method of Mizutani & Wada (1985) and Wada *et al.* (1984), respectively. Isotopic ratios were measured using a Hitachi RMU-6R mass spectrometer and expressed in conventional delta (δ) notation in units of per-mil:

$$\delta X (‰) = \left\{ \frac{R_{sample}}{R_{standard}} - 1 \right\} \times 1,000,$$

where $X = {}^{13}C$ or ${}^{15}N$, and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, respectively. Atmospheric nitrogen (for $\delta^{15}N$) and Peedee belemnite (PDB) (for $\delta^{13}C$) were used as the standards. The precision of isotopic measurements was better than ±0.2‰.

Results and discussion

In estuaries, organic matter in sediment is derived from several major sources: terrestrial plants, salt marsh plants, phytoplankton, and benthic algae. Marine organic matter (phytoplankton) is usually isotopically heavier ($\delta^{13}C$ - 20 ~ -23‰; $\delta^{15}N$ + 6 ~ +11‰) than terrestrial matter (C3 plant: $\delta^{13}C$ - 29 ~ -26‰; $\delta^{15}N$ - 5 ~ +3‰) (Peterson *et al.*, 1985; Craft *et al.*, 1988; Wada *et al.*, 1987b). As a result, $\delta^{13}C$ and $\delta^{15}N$ values in organic matter in estuarine sediments generally increase from the upper reaches to the open sea

(Frey & Sherr, 1984; Wada et al., 1987b; Matson & Brinson, 1990; Thornton & McManus, 1994).

In the Nanakita River Estuary, the sedimentary δ ^{15}N was lighter in the upper estuary (2.0 $\sim$ 3.9‰) and heavier at the river mouth (4.4 $\sim$ 4.7‰) and in the lagoon (4.3 $\sim$ 6.2‰) (Table 1). However, sedimentary organic C exhibited no such trend and had a rather constant δ ^{13}C value (−26.8 $\sim$ −24.4, average -25.6‰) throughout the river estuary, although the sediment δ ^{13}C also tended to increase slightly from the upper estuary to the river mouth and the lagoon (Table 1). This result suggests that terrestrial material is the principal source of carbon in the sediment of this estuary, since the δ ^{13}C value was similar to that of upland plants. In fact, Wada et al. (1993) estimated that land-derived organic matter accounted for c.90% of sedimentary organic matter in the Nanakita River Estuary.

Kikuchi et al. (1992) investigated the phytoplankton and nutrient budgets of the Gamō Lagoon and showed that nutrient fluxes from the sediments support the high phytoplankton production and that the lagoon plays a significant role in the supply of plankton biomass to the surrounding areas. In other words, autochthonous production in the Gamō Lagoon is a significant source of organic matter in the estuary. In the Gamō Lagoon, a fish pond adjacent to the lagoon occasionally discharges a high level of suspended solids with a heavy δ ^{15}N value (9.5‰), which is deposited and acts as another source of sedimentary organic matter in the lagoon (Kikuchi et al., 1992; Wada et al., 1993). Consequently, the sediment δ ^{15}N became heavier in the lagoon and near the lagoon mouth than in the upper estuary as indicated in Table 1, though the sedimentary δ ^{13}C values remained almost constant throughout the estuary. This strongly suggests that the suspended solids with heavy δ ^{15}N from the fish pond causes the heavier δ ^{15}N value in the lagoon and the lower estuary directly or via phytoplankton production.

Stable isotope analysis is one of the most powerful approaches for identifying carbon sources for animals (Haines, 1976; Fry & Parker, 1979; Rodelli et al., 1984), since there is a small fractionation during trophic transfer. The results obtained in this study show that in the upper part of Nanakita River Estuary, a surface deposit-feeding polychaete, N. japonica, had a δ ^{13}C value similar to that of the surrounding sediment (Table 1). This suggests that the carbon of N. japonica and the sediment originated from the same source such as terrestrial plants, judging from the lighter δ

^{13}C values (−26 $\sim$ −25‰) in the upper estuary. In contrast, N. japonica had heavier δ ^{13}C values than the surrounding sediment in the lower river estuary and the Gamō lagoon (t-test, $p < 0.01$). In addition, variations of the stable isotope ratios of the same species of animal collected from the same location are generally very small (standard deviations $\approx$ ±0.5‰) (Mizutani et al., 1992). This indicates that the carbon sources of N. japonica are different between the lower and the upper estuary and suggests that a significant fraction of the polychaete carbon is derived from phytoplankton, which was found to have heavier δ ^{13}C values than the land-derived organic matter in the lower estuary. N. japonica feeds on freshly deposited organic matter, which is also the source of sedimentary organic matter. Vascular plants from land contain large amounts of refractory substances such as cellulose and lignin, while phytoplankton contains high amounts of easily decomposable substances such as protein and carbohydrates which are digestible by invertebrate animals (Kristensen, 1972). During decomposition, these labile compounds are degraded much faster than vascular plants, resulting in accumulation of relatively refractory plant-derived carbon in the sediment. Consequently, the discrepancy in the δ ^{13}C of N. japonica tissue and the surrounding sediment in the lower estuary can be explained as being due to the rapid assimilation of phytoplankton-derived organic matter into the animals and accumulation of plant-derived carbon in the sediment.

The δ ^{15}N of the polychaetes, inhabiting different locations, showed similar values throughout the estuary and were heavier than those of the sediment they inhabited (Table 1). The difference in δ ^{15}N between sediment and corresponding polychaetes was 5 ± 1‰ on the average. In contrast to carbon, heavy (^{15}N) isotope enrichment occurs between animals and their foods, with an enrichment factor of 3 $\sim$ 4‰ per trophic level (Fry & Sherr, 1984; Minagawa & Wada, 1984). Therefore, the discrepancy in the δ ^{15}N of the polychaetes and the surrounding sediment may be explained partly by the enrichment in ^{15}N during trophic transfer. Furthermore, site specific selective utilization of organic nitrogen provided variable δ ^{15}N values for the polychaetes. For example, polychaetes (Notomastus from Sts. A and B) that inhabit the Gamō lagoon and the lower estuary are considered to feed mostly on phytoplankton-derived organic matter with a heavier δ ^{15}N value than the worms in the upper estuary. However, N. japonica found in the upper estuary had similar δ ^{15}N values to the polychaete in the

Table 1. δ ^{13}C and δ ^{15}N of sediment and polychaetes collected in the Nanakita River Estuary and Gamō Lagoon, and the difference in the isotope ratios between concurrently collected sediment and polychaetes at the same location.

Station	Distance from the mouth (km)	δ [13]C (‰)			δ [15]N (‰)		
		Sediment	Polychaetes	Difference	Sediment	Polychaetes	Difference
		NEANTHES			*NEANTHES*		
Upper estuary							
1	3.5	−26.8	−26.4	0.4	3.3	10.4	7.1
2	2.3	−25.3			3.9	9.2	5.3
3	1.6	−26.4	−25.8	0.6	2.0	8.8	6.8
4	0.9	−25.8	−24.2	1.6	2.9	7.5	4.6
Average		−26.1	−25.5[1]	0.9	3.0[2]	9.0	6.0
Lower estuary and lagoon							
5	0.6	−25.7	−22.9	2.8	4.4	9.4	5.0
6	0.35	−25.4	−23.6	1.8	4.7	9.9	5.2
A	1.3	−24.8	−22.7	2.1	4.3	10.0	5.7
B	0.75		−23.0		4.5	10.0	5.5
C	0.6	−24.6	−23.2	1.4	6.2	10.4	4.2
Average		−25.1	−23.1[1]	2.0	4.8[2]	9.9	5.1
		NOTOMASTUS			*NOTOMASTUS*		
Upper estuary							
2	2.3	−25.3	−23.5	1.8	3.9	7.7	3.8
Lower estuary							
5	0.6	−25.7	−22.3	3.4	4.4	9.5	5.1
Lagoon							
A	1.3	−24.8	−21.4	3.4	4.3	11.0	6.7
B	0.75		−21.4		4.5	11.2	6.7

[1] Significant difference between averages at $p < 0.01$,
[2] $p < 0.05$.

lower estuary and the Gamō Lagoon (Table 1). In the upper estuary, the δ ^{15}N values of *N. japonica* and the surrounding sediment were separated by 6‰, corresponding to about two trophic transfers, while their δ ^{13}C values were almost the same. This fact indicates that *N. japonica* in the upper estuary fed on organic matter derived from terrestrial C3 plants as a carbon source, but utilized nitrogen sources other than sedimentary organics, the δ ^{15}N of which was rather light (2 ~ 4‰). The latter contradiction may be explained as follows. Most deposit-feeders are unable to use non-living terrestrial plant material in deposits directly and must rely on microbes to convert the plant tissue to digestible degradation products and easily assimilable microbial biomass which are then available to animals

(Kristensen, 1972; Fenchel & Jorgensen, 1977). During decomposition, the detrital organic matter may be enriched in δ ^{15}N due to microbial transformation of nitrogen (Macko & Zieman, 1983; Wada *et al.*, 1987b; Owens & Law, 1989). The larger isotopic difference in δ ^{15}N between *N. japonica* and the surrounding sediment in the upper estuary may result from selective assimilation of bacterial N or bacterially-altered compounds in the diet.

The δ ^{15}N of *Notomastus* sp. was also heavier than that of the surface sediment they inhabited throughout the estuary (Table 1). Since *Notomastus* sp. is a deep deposit-feeder, it likely feeds on detritus which is older than that favored by surface deposit-feeders. In this study, stable isotopic values of *Notomastus* sp. are

compared with surface (top 1 cm) sediment rather than with sediment from its feeding depth. If it is assumed that detrital organic matter is gradually enriched in ^{15}N due to microbial transformation of nitrogen in sediments (Owens & law, 1989), it might be expected that the deeper sediment would have a more or less heavier δ ^{15}N value than the relatively fresh surface sediment. Consequently, the isotopic difference in δ ^{15}N between *Notomastus* and the surrounding surface sediment may be explained as being due to *Notomastus* ingestion of the deeper sediment which is isotopically heavier than the surface sediment, as well as to the selective assimilation of bacterially-altered compounds in the diet. However, only the top 1 cm of sediment was measured in the present study. Further detailed examination of the vertical profiles is henceforth required.

The δ ^{13}C results indicate that *Notomastus* sp. was enriched in ^{13}C relative to surrounding surface sediments (Table 1) and had heavier δ ^{13}C values than *N. japonica* in the same location (t-test, $p < 0.06$). *Notomastus* sp. is a deep deposit-feeding polychaete which obtains carbon from deep sediment that is assumed to be depleted of labile carbon relative to surface sediment. It can be assumed that mud-feeders selectively assimilate only the labile organic fractions (Kristensen, 1972). Similar enriched values have been found in deep deposit-feeding capitellid polychaetes, *Capitella* spp. (Spies *et al.*,1989) and *Heteromastus filiformis* (Clough & Lopez, 1993). Selective utilization of sedimentary carbon by animals following bacterial fractionation might then alter the stable isotope ratio from the original carbon source. Clough & Lopez (1993) suggested the possibility that enriched δ ^{13}C values of *H. filiformis* reflected partial incorporation of bacterial carbon derived from CO_2 and dissolved compounds in overlying sea water into the worms. The enrichment of heavy isotope in deposit-feeding animals requires further investigation.

References

Clough, L. M. & G. R. Lopez, 1993. Potential carbon sources for the head-down deposit-feeding polychaete *Heteromastus filiformis*. J. mar. Res. 51: 595–616.

Craft, C. B., S. W. Broome, E. D. Seneca & W. J. Showers, 1988. Estimating sources of soil organic matter in natural and transplanted estuarine marshes using stable isotopes of carbon and nitrogen. Estuar. coast. Shelf Sci. 26: 633–641.

DeNiro, M. J. & S. Epstein, 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta 42: 495–506.

Fenchel, T. M. & B. B. Jorgensen, 1977. Detritus food chains of aquatic ecosystems: The role of bacteria, In Advances in microbial ecology. Plenum: 1–58.

Fry, B. & P. L. Parker, 1979. Animal diet in Texas seagrass meadows: ^{13}C evidence for the importance of benthic plants. Estuar. coast. mar. Sci. 8: 499–509.

Fry, B., A. Joern & P. L. Parker, 1978. Grasshopper food web analysis: use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. Ecology 59: 498–506.

Fry, B. & E. B. Sherr, 1984. δ ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. mar. Sci. 27: 13–47.

Haines, E. B., 1976. Stable carbon isotope ratios in the biota, soils and tidal water of a Georgia salt marsh. Estuar. coast. mar. Sci. 4: 609–616.

Haines, E. B. & C. L. Montague, 1979. Food sources of estuarine invertebrates analyzed using $^{13}C/^{12}C$ ratios. Ecology 60: 48–56.

Hanawa, K. & T. Sugimoto, 1979. Effect of the variation of river discharge on flushing and recovery of salt wedge. Sci. Rep. Tohoku Univ. Ser. 5 Geophysics 25: 219–233.

Kikuchi, E., 1987. Effects of the brackish deposit-feeding polychaetes *Notomastus* sp. (Capitellidae) and *Neanthes japonica* (Izuka) (Nereidae) on sedimentary O_2 consumption and CO_2 productin rates. J. exp. mar. Biol. Ecol. 114: 15–25.

Kikuchi, E., G. Urano & Y. Kurihara, 1992. The use of dialysis culture in a study of chlorophyll budget in a brackish lagoon, Japan. Hydrobiologia 245: 1–13.

Kristensen, J. H., 1972. Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. Mar. Biol. 14: 130–142.

Macko, S. A. & J. Zieman, 1983. Stable isotope composition and amino acid analysis of estuarine plant litter undergoing decomposition [abstract]. Estuaries 6: 304.

Matson, E. A. & M. M. Brinson, 1990. Stable carbon isotopes and the C:N ratio in the estuaries of the Pamlico and Neuse Rivers, North Carolina. Limnol. Oceanogr. 35: 1290–1300.

Minagawa, M. & E. Wada, 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between δ ^{15}N and animal age. Geochim. Cosmochim. Acta 48: 1135–1140.

Mizutani, H. & E. Wada, 1985. Combustion of organic samples by infrared furnace for carbon isotope analysis. Analyt. Biochem. 146: 90–95.

Mizutani, H., M. Fukuda & Y. Kabaya, 1992. ^{13}C and ^{15}N enrichment factors of feathers of 11 species of adult birds. Ecology 73: 1391–1395.

Owens, N. J. P. & C. S. Law, 1989. Natural variations in ^{15}N content of riverine and estuarine sediments. Estuar. coast. Shelf Sci. 28: 407–416.

Peterson, B. J. & R. W. Howarth, 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnol. Oceanogr. 32: 1195–1213.

Peterson, B. J., R. W. Howarth & R. H. Garritt, 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science 227: 1361–1363.

Rau, G. H., 1981. Low $^{15}N/^{14}N$ of hydrothermal vent animals: Ecological implications. Nature 289: 484–485.

Rodelli, M. R., J. N. Gearing, P. J. Gearing, N. Marshall & A. Sasekumar, 1984. Stable isotope ratio as a tracer of mangrove carbon in Malaysian ecosystems. Oecologia 61: 326–333.

Sayama, M. & Y. Kurihara, 1983. Relationship between burrowing activity of the polychaetous annelid, *Neanthes japonica* (Izuka) and nitrification-denitrification processes in the sediments. J. exp. mar. Biol. Ecol. 72: 233–241.

Simenstad, C. A. & R. C. Wissmar, 1985. δ ^{13}C evidence of the origins and fates of organic carbon in estuarine and nearshore food webs. Mar. Ecol. Prog. Ser. 22: 141–152.

Spies, R. B., J. E. Bauer & D. Hardin, 1989. Stable isotope study of sedimentary carbon utilization by *Capitella* spp.: effects of two carbon sources and geochemical conditions during their diagenesis. Mar. Biol. 101: 69–74.

Thayer, G. W., P. L. Parker, M. W. LaCroix & B. Fry, 1978. The stable carbon isotope ratio of some components of an eelgrass, *Zostera marina*, bed. Oecologia 35: 1–12.

Thornton, S. F. & J. McManus, 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: evidence from the Tay estuary, Scotland. Estuar. coast. Shelf Sci. 38: 219–233.

Wada, E., H. Mizutani, K. Karasawa, Y. Kabaya, M. Minagawa, S. Yonemoto & T. Tsuji, 1984. Carbon and nitrogen isotopic composition as tracers of transport of organic matter along the Otsuchi watershed. Chikyukagaku 18: 89–98.

Wada, E., M. Terazaki, Y. Kabaya & T. Nemoto, 1987a. ^{15}N and ^{13}C abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. Deep-Sea Res. 34: 829–841.

Wada, E., M. Minagawa, H. Mizutani, T. Tsuji, R. Imaizumi & K. Karasawa, 1987b. Biogeochemical studies on the transport of organic matter along the Otsuchi River watershed, Japan. Estuar. coast Shelf Sci. 25: 321–336.

Wada, E., Y. Kabaya & Y. Kurihara, 1993. Stable isotopic structure of aquatic ecosystems. J. Biosci. 18: 483–499.