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Trophic relations between animal and non-animal matter in sedements investigated using stable isotopes

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Abstract — Three sets of sediment traps were placed in Otsuchi Bay, Northeast of Japan, one near the shore at 2 m depth, two at the center of the bay at 2 m and 13 m depth each. Collections were made at least once a month, from 2003/12 to 2005/06. dS, dC and dN stable isotope values of the collected material were measured. Some samples contained visible animals, especially near the shore in spring, but animals were caught all year round in all sediment traps. There was a seasonal variation in the results, so the samples were divided into two kinds: samples from spring and summer, and samples from autumn and winter. Samples from the center of the bay at 2 m and 13 m in depth were very similar, so they were grouped together. In most of the cases, the samples containing animals had dC and dS similar to samples without animals, an indication that they have the same origin, except for samples from the center of the bay, especially in autumn and winter. The dN values of samples containing animals were always higher than those of samples without animals, except for near the shore in autumn and winter, with exceptionally low dN values for samples containing animals. It was assumed that the sedimentary organic matter reflected the isotopic composition of particulate organic matter (POM), and it was hypothesized that a great portion of the POM from near the shore might have been the main food source of the animals. If so, at the center of the bay, especially in autumn and winter, edible food was scarce, and the animals would be there for other reasons that not foraging. If the analyzed animals can be thought as representative of the primary consumers of the water column in the bay, then a part of POM would not enter the pelagic food chain.

Key words: stable isotopes, organic sediment, trophic relations

Introduction

Sediment trap is widely used to collect the sedimentary matter in a water body. The organic portion of the sedimentary material comes from the particulate organic matter (POM), which is assumed to be the food source of the primary consumers in water column, and even for those from the bottom. There are several primary producers that can supply POM to coastal areas and estuaries, so that it is difficult to know exactly what it comprises in these ecosystems. Consequently, it becomes difficult to know to what extent the primary consumers use the POM as food source. Incomplete knowledge on their feeding habits in natural habitat renders it more difficult to study the system.

Stable isotopes have been proven to be a useful tool in dealing with trophic transfer of materials (Zieman et al. 1984, Peterson 1999, Navandi and Dworschak 2005). The main feature of the technique is that the stable isotopes of the chemical elements involved in trophic processes (as C, N, H, O and S) can give information not only on these developments, but also on elements' sources (Peterson and Fry 1987). Compared to traditional methods of food web analysis (gut content analysis, direct observation in field or in laboratory, radiotracer techniques), stable isotopes have the advantages of simple sample collection, easiness of analysis procedures, and a progressive tendency of cost lowering as automation becomes more prevalent (Michener and Schell 1994).

Among those chemical elements whose stable isotopes can be used in food web studies, C, N and S have been the most commonly used. Each of them has its own strength and weakness, but, if used together, they can provide a more significant power to resolve food web structures (Peterson et al. 1985).

The objective of this study is to investigate if the animals found in sediment traps used the POM as their main source of food by comparing their C, N and S stable isotopic composition to that of the sedimentary non-animal matter. To date, the composition of the sedimentary matter and feeding habits of the animals are not precisely known, but their stable isotopic composition may provide us clues on the trophic relations between animals and the POM.

Materials and Methods

Three sets of sediment traps (Table 1) were placed in Otsuchi bay (Fig. 1) as follows: one near the shore (station A), and two at the center of the bay (station B). The former was set at a depth of 2 m, while the others were at 2 m and 13 m. The period of study extended from December 2003 to June 2005.

The traps were left in the bay for one or two weeks, approximately. After the collection of the traps, their contents were transported to the laboratory, where they were centrifuged to separate the particulate matter from the water. The particulates were weighed and a part of the mass was dried until constant weight at 60°C for dry weight calculation. Another part was stored in a 5% Formalin solution for subsequent observation. The dried material was ground with a mortar and a pestle, and stored in a desiccator until stable isotope analysis.

Visual inspection of the dried samples was performed for preliminary determination of the composition of each sample. Some of the samples contained whole or broken animal bodies (referred as samples containing animals, SCA), while others did not (referred as samples without animals, SWA). The visible animal matter of SCA was not separated from the material that could not be visually identified as of animal origin.

Stable isotope analysis was performed to calculate $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ ratios with an elemental analyzer (Flash EA 1112) coupled with a mass spectrometer (DELTA plus XP) by a ConFlo III interface. For sulfur, samples were placed in tin crucibles and led to the analyzer directly. For carbon and nitrogen, which were measured together, samples were put in silver crucibles, few drops of a solution of HCl : H_2O (1 : 1) were added to remove the carbonates and samples were dried at 80°C, before they were placed in tin crucibles. Once in the elemental analyzer, samples were burn. The resulting gases from combustion (N_2 and CO_2 for Carbon and Nitrogen, SO_2 for sulfur) had their stable isotopic ratios measured with the mass spectrometer. Blank tests were conducted, with crucibles containing only the acid solution, in order to verify if the acid solution and the crucibles would interfere with the analysis; there was no detectable response.

The stable isotopic composition of gasses used as reference by the mass spectrometer was not determined, hence, it was necessary to calibrate the measurements using the following chemicals as standards: L-Cystin, Sulfanilamide and Ag_2S for sulfur ratio; L-Alanin for carbon and nitrogen ratios. The precision of the analyses was 0.5‰ for sulfur ratio, and 0.15‰ for carbon and nitrogen ratios.

The results for the stable isotopic analyses are shown in the following notation, dE, which comes from:

$$dE=[(R_{sample}-R_{reference})-1]-10^3‰$$

Table 1. Characteristics of sediment traps.

Height	49 cm
Diameter	14.5 cm
Recoverable volume	1.5 L
Grid mesh size	2 cm
Number of traps per set	2

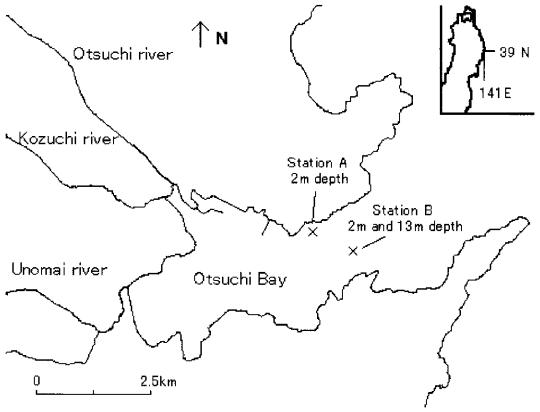

Fig. 1. Location of the sediment traps inside the bay.

Where:

dE is the value for the element E.

R is the ratio, which is $^{13}C/^{12}C$, for carbon, $^{15}N/^{14}N$, for nitrogen, and $^{34}S/^{32}S$, for sulfur.

Sample refers the analyzed sample.

References used were very known substances: Pee Dee belemnite for carbon, atmospheric nitrogen for nitrogen and Canion Diablo troilite for sulfur.

Results

Most of the animals were found in the sediment collected at station A in spring. At station B, the animal frequency was higher at 13 m depth than at 2 m. Among the observed animals, the most common were isopods, amphipods and shrimps.

After a scrutiny of the stable isotopic measurement results, we pooled the samples in homogeneous groups according to their values (Tables 2 for SWA, Table 3 for SCA). For SWA, the stable isotopic ratios' means±SD (dC=−19.7±0.8, dN=5.4±0.8 and dS=19.0±1.5) were higher than those from autumn and winter at the same point (dC=−20.8±1.1, dN=4.3±1.0 and dS=16.5±2.0) at station A in spring and summer. The values of SWA at station B at different depths showed similar variation along the time (Fig. 2; no statistically significant difference at $\alpha \leq 5\%$, Student t test), so they were grouped together. Their values varied seasonally: very low in autumn and winter (dC=−22.8±0.8, dN=3.3±0.8 and dS=12.5±3.0); very high dC values observed in April, early spring: dC=−18.6±0.5, dN=4.7±0.7 and dS=18.0±

Table 2. Stable isotopic values of samples without animals. Mean±SD (number of samples). Same letters in the same column show non-significant mean difference (one-way ANOVA, Tukey–Kramer test, $\alpha \leq 5\%$).

Station–Time	dC	dN	dS
A–spring and summer	−19.7±0.8(27)	5.4±0.8(27)a	19.0±1.5(28)a
A–autumn and winter	−20.8±1.1(33)a	4.3±1.0(33)b	16.5±2.0(32)
B–April	−18.6±0.5(24)	4.7±0.7(24)bc	18.0±1.5(24)a
B–May to September	−21.3±0.8(29)a	5.0±0.8(29)ac	18.5±2.0(29)a
B–autumn and winter	−22.8±0.8(50)	3.3±0.8(50)	12.5±3.0(47)

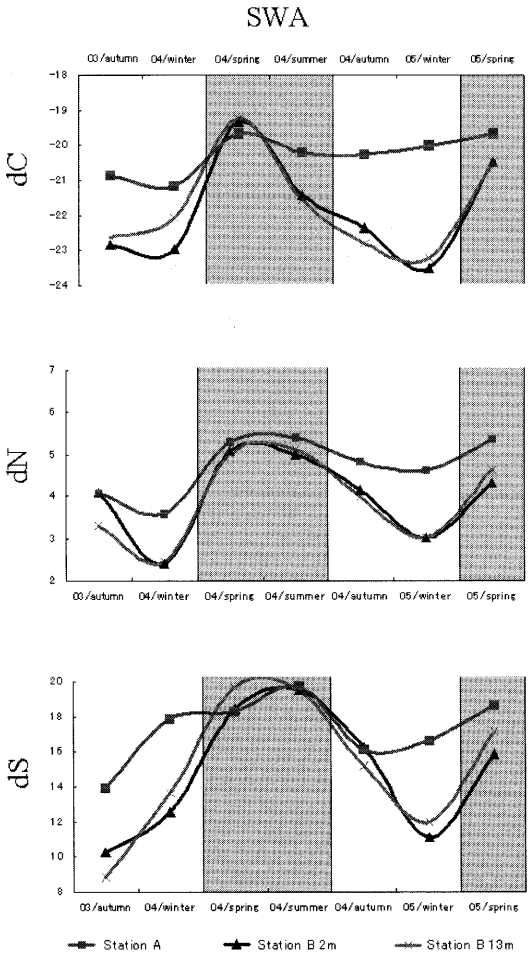

Fig. 2. Stable isotopic values of samples without animals from the center of the bay along time.

1.5; intermediate values from May to September, late spring and summer: dC=−21.3±0.8, dN=5.0±0.8 and dS=18.5±2.0.

One-way ANOVA was executed to verify the similarities among SWA from different spatial-temporal situations (Table 2). For dC, only station A in autumn and winter and station B in April were not significantly different. For dN, there were three pairs of samples not significantly different: 1) station A in spring and summer, station B in autumn and winter; 2) station A in autumn and winter, station B in April; 3) station B in April and station B from May to September. For dS, three groups were not significantly different: station A in spring and summer, station B in April, and station B from May to September.

SCA showed a comparable behavior to SWA at station A, that is, higher values in spring and summer (dC=−19.9±0.8, dN=6.3±1.1 and dS=18.5±1.0), and lower values in autumn and winter (dC=−20.0±1.2, dN=4.7±1.1 and dS=17.5±3.0). However, SCA had higher dN than SWA at station A.

At station B, in spring and summer, SCA from 2 m and 13 m showed similarity in their stable isotopic values (2 m: dC=−20.2±1.0, dN=6.2±0.8 and dS=18.0±1.5; 13 m: dC=−20.5±0.8, dN =5.9±0.8 and dS=18.0±1.5; no statistically significant difference at $\alpha \leq 5\%$, Student t test), so they were grouped together (dC=−20.4±0.9, dN=6.0±0.8 and dS=18.0±1.5). No SCA could be found in April in station B, so the division done for SWA from station B in spring and summer was not done for SCA. As the frequency of SCA from 2 m depth in autumn and winter was very small (4 of 17 samples for dS, 1 of 13 samples for dC and dN), they were grouped together with samples from 13 m depth, making one set of data (dC=−19.4±1.9, dN=6.0±1.2 and dS=17.0±2.0).

One-way ANOVA was executed to verify the similarities among SCA from different spatial-temporal situations (Table 3). Except for dN at station A in autumn and spring, SCA had a more or less constant stable isotopic composition along the time and space.

SWA and SCA were compared at each spatial-temporal situation (Table 4). For station B in spring and summer, SCA were compared with SWA from April and with SWA from May to Spetember. dC was not statistically different between SWA and SCA at Station A, and significantly different at Station B. dN was significantly different between SWA and SCA in all occasions, except at Station A in autumn and winter. dS of SWA and SCA were not significantly different in all occasions, except for station B in autumn and winter.

Table 3. Stable isotopic values of samples containing animals. Mean±SD (number of samples). Same letters in the same column show non-significant mean difference (one-way ANOVA, Tukey–Kramer test, $\alpha \leq 5\%$).

Station–Time	dC	dN	dS
A–spring and summer	−19.9±0.7(18)a	6.3±1.1(18)a	18.5±1.0(18)a
A–autumn and winter	−20.0±1.2(11)a	4.7±1.1(11)	17.5±3.0(11)a
B–spring and summer	−20.4±0.9(20)a	6.0±0.8(20)a	18.0±1.5(18)a
B–autumn and winter	−19.4±1.9(13)a	6.0±1.2(13)a	17.0±2.0(17)a

Table 4. Comparison between samples without animals and samples containing animals for each considered spatial-temporal situation. SCA from station B in spring and summer were compared to SWA from April and to SWA from May to September.

Station–Time	dC	dN	dS
A–spring and summer	NS	+	NS
A–autumn and winter	NS	NS	NS
B–April	+	+	NS
B–May to September	+	+	NS
B–autumn and winter	+	+	+

+: Significant difference at $\alpha \leq 5\%$. Student t test.
NS: Non-significant difference at $\alpha \leq 5\%$. Student t test.

Discussion

One important assumption for our study is: the POM has stable isotopic composition equal to that of sedimentary organic matter, despite they might have been different materials. The phytoplankton, in some instances, is not expected to sink; instead, copepod feces sink (Longhurst, 1998). However, since copepods eat phytoplankton, their feces are expected to have similar dC and dN values to their food. Gorokhova and Hansson (1999) showed that *Mysis mixta* had feces with similar dC and dN values of their recently ingested foods. dS may have the same characteristic.

If it is desired to know the trophic relation between consumers and foods in a trophic chain by the use of stable isotopes, the key variable is the stable isotopic trophic shift, indicated by ΔdE, for the element E, that is the difference between the stable isotopic value of the consumer and its food. The most recent comprehensive survey on the subject was reported by McCutchan et al. (2003), indicating $\Delta dC = 0.3 \pm 0.14‰$ and $\Delta dS = 0.5 \pm 0.56‰$. In our investigation, however, instead of using these precise estimations, ΔdC and ΔdS were assumed as approximately equal to zero. For these results, the following reasons are considered: 1) The components of SWA were potentially highly variable, with both eatable and non eatable matter mixed in variable proportions in each sample; 2) It was not known the proportion of animal mass in each SCA sample, a constant difference in isotopic values between SCA and SWA could not be expected; 3) Trapped animals were pooled together without any classification, so small differences in their feeding habits were masked.

For dN, matters are more complicated, because McCutchan et al. (2003) observed that ΔdN changes significantly according to species, diet and animal condition. Besides, Adams and Sterner (2000) pointed that it is strongly necessary to further study the relationship between organismal dN values and dietary nutritional contents. So, the most appropriate approach for our research was to use ΔdN values from reports of situations that share similarity with those found here.

Beginning with the samples from spring and summer at station A, dC and dS were not significantly different between SCA and SWA (Table 4). This would mean that it is possible for a trophic relation to exist between the animals of SCA and the POM (represented by SWA), because ΔdC and ΔdS would be around 0. If so, would ΔdN from SWA to SCA (significantly different from 0; in average, equal to 0.8) be a possible indication that the animals eat the POM? In the study of Macko et al. (1982), amphipods feeding on macroalgae showed a ΔdN of −0.7‰ or 2.3‰, depending on the animal species, which encourages us to believe that the ΔdN found here can be possible. The work of Vander Zanden and Rasmussen (2001) would also support our hypothesis: a ΔdN between 0 and 1 could be observed in invertebrates, marine animals or herbivores, that is, the probable characteristics of the animals found here.

Stable isotopic values of SCA from station B in spring and summer were very similar to those from station A in the same period (Table 3). This may mean that the animals from both stations relied on the same food, which seemed to be the POM present at station A. Stable isotopic values of SWA from April reflected a pulse of material that left its stable isotopic signature, probably the spring bloom of phytoplankton, that has been observed in Otsuchi bay (Furuya et al. 1993) and in other bays of the region (Enoki 2002) to occur in April. This signature disappeared quickly already in the following month. Concerning the animals' food source, it seems that the POM from station B did contain food for the animals, but in a smaller proportion than those from station A in spring and summer, because only dS was not statistically different between SCA and SWA (Table 4).

SWA from station A in autumn and winter had a quite

different stable isotopic composition compared to those from spring and summer, showing that their materials came from different sources (Table 2). The present animals seemed to rely on the present POM, because of the ΔdE (not significantly different from 0 for the tree stable isotopes, Table 4) was comparable to the situation of spring and summer near the shore, except for dN. Anyway, this ΔdN would still be supported by the results of Macko et al. (1982). The animals caught at station A in spring and summer were in general of the same species of those caught in autumn and winter, which could imply that a diet shift in the life cycle of these organisms occurred, following the available food.

The low dC, dN and dS values of SWA from station B in autumn and winter show clearly that their origin is distinct from the other SWA (Table 2). The stable isotopic composition of the present SCA was very similar to that of SCA from spring and summer (Table 3), which suggests that the animals of both sets of samples ate the same food. Then, two situations can be imagined: 1) the available food for the animals was scarce, and this would indicate that the animals found at the center of the bay were not foraging, because the SWA from near the shore seemed to be a better resource; 2) the animals shifted their diet to the present POM, but this could not be seen from the stable isotopic values. The later was the case in Fry and Arnold (1982), in which shrimps needed to at least quadruplicate their weight before a change in their dC could be observed. According to McCutchan et al. (2003), this characteristic of dC is probably valid for dN and dS also. However, we are more inclined to believe in the first hypothesis, that is, the animals experienced scarcity of food at the center of the bay in autumn and winter, because the animals found at station A were generally the same of station B, and at station A the SCA were similar to SWA in autumn and winter.

In general, the trophic situations near the shore and at the center of the bay were different: the proportion of POM assimilated by the animals was higher at station A than at Station B. If we take the analyzed animals as representative of the primary consumers of the water column, it would imply that a portion of the sedimentary matter is not incorporated in the pelagic food chain. The following research assesses the origin of the sedimentary matter, and it should give light on which material is incorporated or not in the pelagic food chain of Otsuchi bay. If this objective is accomplished, it will be possible to know the most important material sources for the organisms of the bay. As a consequence, the importance of the dependence on land originated material for fisheries will be nearer of having a conclusive answer.

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