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MEIOBENTHOS
INGESTION RATE
DEEP SEA
ENERGY BUDGET

ABSTRACT – Ingestion rates of bathyal deep-sea meiobenthos collected from Suruga Bay, central Japan, were measured using either ^{14}C-amino acid mixture or ^{3}H-thymidine as radioactive tracers. When ^{14}C-amino acid mixture was used, active incorporation of the tracer by the meiofauna was not detectable. On the other hand, if ^{3}H-thymidine was used, labels of specimen in control were constant, whereas those in feeding experiments increased with the meiofaunal body mass. Ingestion rate of a meiofaunal individual of 1 nl in body mass was estimated as 3.51 nl of sediment/hr assuming non-selective deposit feeding. This result is enigmatic because it means the animal feeds on 3.5 times more volume of sediment than its own body mass every hour. Assuming selective feeding on bacteria, the ingestion rate was estimated as 18,900 cells/hr, or 0.378 ng of C/hr. This value is close to the estimated energy consumption through respiration by the deep-sea meiofauna.

MEIOBENTHOS
TAUX D'INGESTION
ZONE BENTHIQUE PROFONDE
BUDGET ÉNERGÉTIQUE

RÉSUMÉ – Les taux d'ingestion du méiobenthos de la zone bathyale profonde récolté dans la Baie de Suruga, Japon Central, ont été mesurés à l'aide de mélanges C^{14}-amino-acide ou de H^{3}-thymidine comme traceurs radioactifs. L'utilisation de mélanges C^{14}-amino-acide ne permet pas de détecter l'incorporation active du marqueur. D'autre part, avec la thymidine H^{3}, le marquage des spécimens témoins reste constant, tandis que celui de la méiofaune en expérience croit avec le poids du corps. Le taux d'ingestion par individu de la méiofaune d'un poids du corps de 1 nl est estimé à 3,51 nl de sédiment à l'heure pour des mangeurs de dépôts non sélectifs. Ce résultat est énigmatique car il signifie que l'animal se nourrit d'un volume de sédiment égal à plus de 3,5 fois son poids à l'heure. Si la nutrition est sélective chez les Bactéries, le taux d'ingestion peut être estimé à 18 900 cellules/heure ou 0,378 ng C à l'heure. Cette valeur est proche de la consommation d'énergie due à la respiration par la méiofaune profonde.

INTRODUCTION

The deep sea is an energy-limited environment (Thiel, 1975, 1979; Shirayama, 1983, 1984). Thus one of the most important subjects in the deep-sea biology is understanding how the organisms have adapted to such a severe environment. Extremely low respiration rates of deep-sea fishes suggest that conserving energy by reducing metabolic activities is one way to adapt to the deep-sea condition (Smith and Hessler, 1974; Smith and Brown, 1983). The reported respiration rates of meiofauna are, however, similar to those of shallow-water relatives (Shirayama, 1992). These results make it questionable as to whether or not deep-sea meiofauna ingest foods as actively as their shallow-water counterparts even though the former inhabit an energy-limited environment.

The technique of measuring ingestion rates of meiofauna using radioactive tracers was developed by Montagna (1984 a, b), and has been applied in several studies of shallow-water meiofauna (Carman, 1990 a, b; Carman and Thistle, 1985). Shirayama (1991) used the technique to measure the ingestion rate of a single species of deep-sea nematode, and emphasized the importance of dissolved organic matter in addition to the particulate organic matter as an energy source for the nematode. The study involved only one species of nematode, but in this study, ingestion rates were measured for not only nematodes but also harpacticoid copepods and polychaetes. In addition, the size range of organisms was extended from an order of 0.1 µg to more than 100 µg. On the basis of the results, energy balance of deep-sea meiofauna will be discussed.

MATERIALS AND METHODS

Deep-sea sediment was collected using a box corer of USNEL type (Hessler and Jumars, 1974) modified to collect 1/10 m^2 of the sediment (Rigosha Co., Tokyo). The sampling was carried out once on November 1, 1986 at station A3 of R/V Tansei Maru cruise KT8601. The sampling station was established at the bay head of Suruga Bay, Central Japan (35°00.96'N 138°41.18' E) at a depth of 1214 m (Fig. 1).

Immediately after hauling up the corer on the board, five subcores were taken by inserting plexiglass tubes (34 mm inner diameter and 300 mm long) into the sediment. The topmost 1 cm layers of the sediment in these subcores were transferred into glass vials separately and 10 ml of the overlying water was added into each vials gently such that the disturbance of the sediment is minimal.

One of these vials was used to determine the abundance of bacteria in the sediment. To fix bacteria, 20 ml of 10 % V/V neutralized formalin was added into the vial on board, and kept refrigerated (5°C). In the laboratory on land, six replicates of 1 ml of the material in the vial were taken out, diluted 10 to 10 000 times with filtered seawater and 0.1 ml of the material was filtered using nucleopore filter of 0.2 μm opening, which had been stained using Irgalan Black. The filter was then stained using DAPI, and the number of bacteria on the filter was counted under a fluorescent microscope.

The sediments in the other glass vials were kept refrigerated at ambient temperature (5°C), and transferred to the radioisotope laboratory on land. Tracer experiments were carried out 48 hours after the collection. I basically followed the method described in Montagna (1984 a) with several modifications. Followings are the brief description of the present method. Into the glass vials which contain the deep-sea sediment, [U-^{14}C] L-amino acid mixture (ICN Radiochemicals, specific activity : 65 mCi/mmole) or [Methyl-^{3}H] Thymidine (ICN Radiochemicals, specific activity : 1.89 mCi/mg) was inoculated so as the radioactivities will be 0.526 and 9.85 μCi/ml, respectively, and the overlying water was stirred so gently as to avoid disturbance of the sediment surface. After culturing at ambient temperature (5°C) for 4.5 hours, 20 ml of 10 % V/V neutralized formalin in seawater was added into each vial and stirred vigorously so as to make the content of the vial uniform in order to stop the experiment. In the control experiments, addition of formalin was done immediately after the inoculation of radioactive tracer.

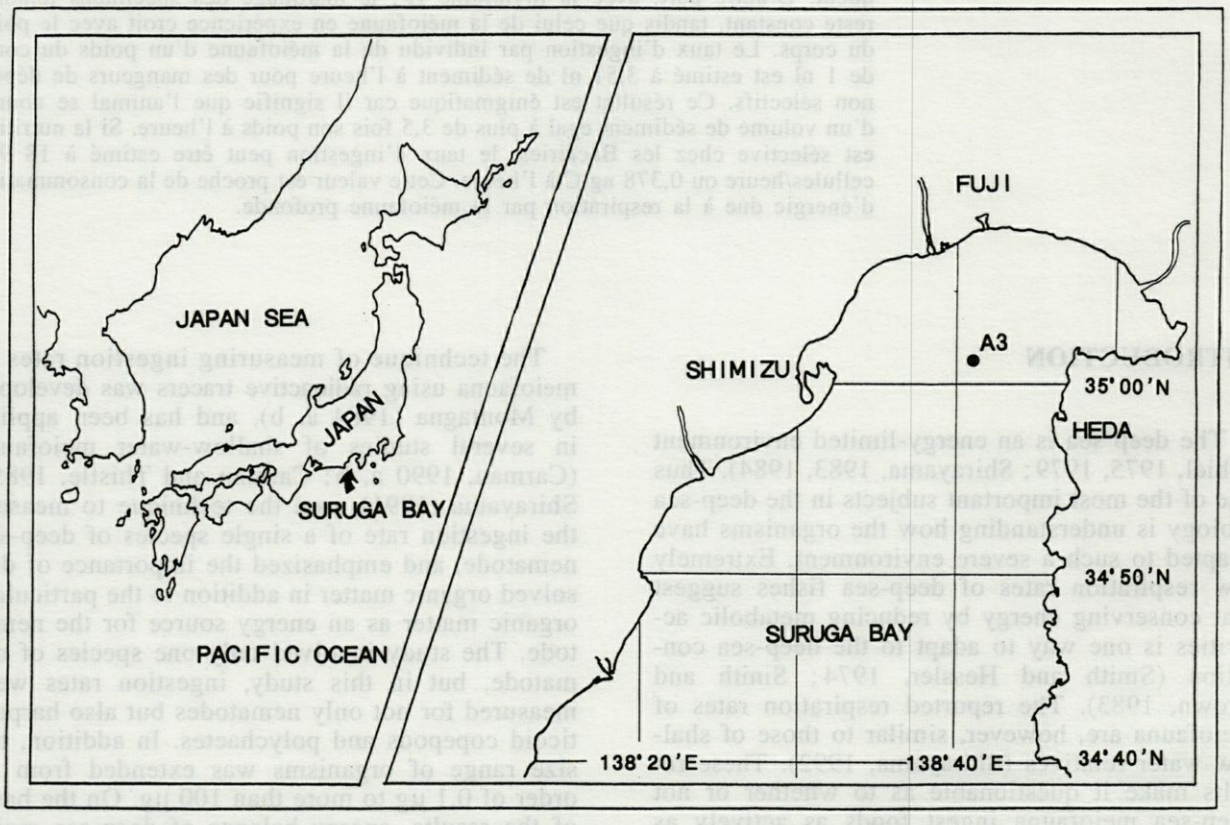

Fig. 1. – Map of sampling station.

To measure the radioactivity of the sediment, nine replicates of 10 µl of the fixed material were taken out from each vial and filtered using millipore filters of 0.2 µm opening. Each filter was washed with seawater, air dried, and placed in a vial for liquid scintillation counting. By adding the liquid scintillator (Aquasol II, Packard) into the vial, the filter became translucent, and the radioactivity of the filter was measured using a liquid scintillation counter (LKB 1215).

The rest of the sediment which had been used for the tracer and the control experiments was washed in a sieve of 63 µm opening, and from the material retained on the sieve, meiofaunal organisms were sorted out under a dissecting microsocope. Each meiofaunal individual was washed with seawater, and its body volume was measured following the method of Warwick and Price (1979). Then it was placed in a vial for liquid scientillation counting, dissolved using 1 ml of Soluence 100s (Packard), and its radioactivity was measured using the liquid scintillation counter (LKB 1215).

RESULTS

The surface sediment was mud, but at the layer of about 3 cm deep, it suddenly changed to coarse sand, suggesting that the area may have been affected by a turbidity current recently. Even though the environment seems to be unstable, a variety of meiofauna was present, and like other meiofaunal communities, nematodes were the most dominant whereas polychaetes and harpacticoids were the subdominant taxonomic groups. In the present study, the ingestion rates of these three major taxonomic groups were measured.

The results of tracer experiments differed depending on the species of tracer. When ^{14}C-amino acid mixture was used as a tracer, label of meiofauna increased with body mass of meiofauna in both control and feeding experiments (Fig. 2A). In this case, regressions for control and experiments were not significantly different, and active ingestion of meiofauna on sediment was not detectable.

In the ^{3}H-thymidine experiment, the radioactivities of meiofauna were constant regardless of body size in the control experiment (Fig. 2B). On the other hand, the label increased significantly with increase of body weight in the feeding experiment. Thus, the average of all results obtained in the control experiments was considered as spontaneous absorption of ^{3}H-thymidine by meiofauna. After substituting this average value from all the experimental results, the following significant regression ($r = 0.64$, $n = 26$, $p < 0.001$) was obtained.

$$\log DPM = 0.144 \log Wt + 0.051 \quad (1)$$

where *DPM* denotes the label of meiofauna (Decay/min) and *Wt* the weight of meiofauna (µg). Assuming that the specific density of meiofauna is 1.13, the above equation was converted to

$$\log DPM = 0.144 \log V + 0.059 \quad (2)$$

where *V* (nl) denotes body mass of meiofauna.

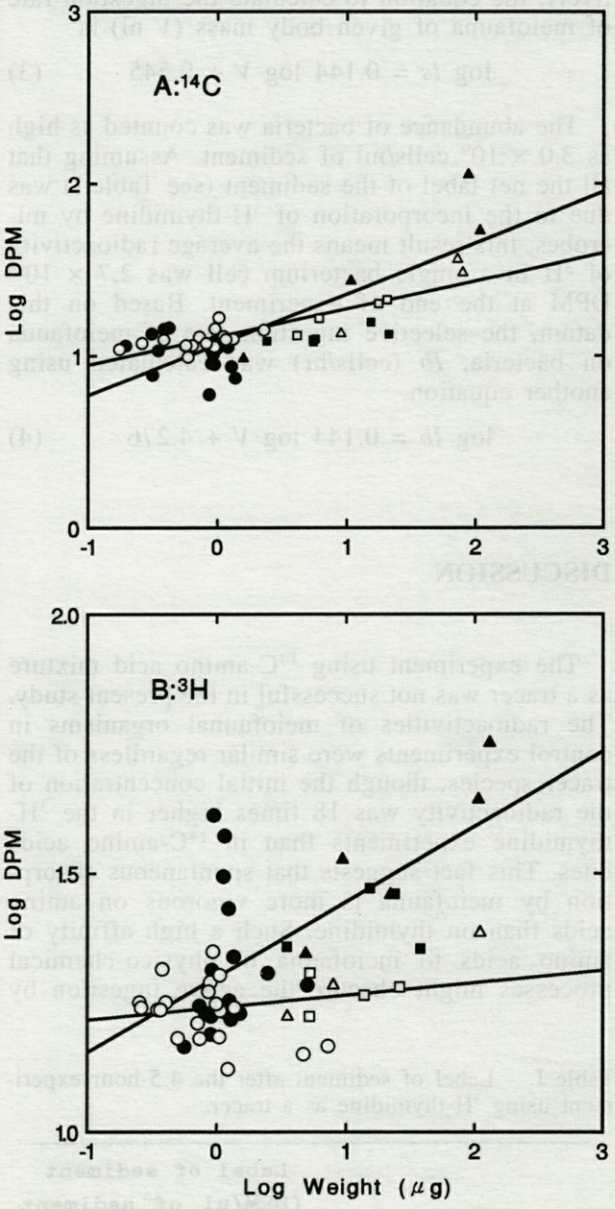

Fig. 2. – Relationships between label of meiofauna and body weight. Two species of radioactive material (A : ^{14}C-amino acid mixture ; B : ^{3}H-thymidine) were used as tracer. Filled and open symbols denote experimental and control data, respectively. Circle, square and triangle symbols denote nematode, copepod and polychaete data, respectively.

After the 4.5-hour experiment, the sediment used for the feeding experiment incorporated ^{3}H-thymidine significantly more than that for the control one (Table I), suggesting active growth of microbes in the present sediment, even though they experienced rapid decompression when sampled from the deep sea.

Ingestion rates of meiofauna on sediment, *Is* (nl of sediment/hr), were calculated based on the label of ^{3}H in sedimentary particles (see Table I) using the formula described in Montagna (1984 a). If the animals fed on the sediment non-selectively, the equation to calculate the ingestion rate of meiofauna of given body mass (V nl) is

$$\log Is = 0.144 \log V + 0.545 \quad (3)$$

The abundance of bacteria was counted as high as 3.0×10^{9} cells/ml of sediment. Assuming that all the net label of the sediment (see Table I) was due to the incorporation of ^{3}H-thymidine by microbes, this result means the average radioactivity of ^{3}H in a single bacterium cell was 2.7×10^{-5} DPM at the end of experiment. Based on this datum, the selective ingestion rate of meiofauna on bacteria, *Ib* (cells/hr) was calculated using another equation,

$$\log Ib = 0.144 \log V + 4.276 \quad (4)$$

DISCUSSION

The experiment using ^{14}C-amino acid mixture as a tracer was not successful in the present study. The radioactivities of meiofaunal organisms in control experiments were similar regardless of the tracer species, though the initial concentration of the radioactivity was 18 times higher in the ^{3}H-thymidine experiments than in ^{14}C-amino acids ones. This fact suggests that spontaneous absorption by meiofauna is more vigorous on amino acids than on thymidine. Such a high affinity of amino acids to meiofauna in physico-chemical processes might obscure the active ingestion by the meiofauna on the particulate matter labelled by ^{14}C-amino acids.

Table I. – Label of sediment after the 4.5-hour experiment using ^{3}H-thymidine as a tracer.

	Label of sediment (DPM/µl of sediment ± S.D.)
Control	64.56 ± 6.76
Feeding Experiment	145.48 ± 20.85
Net incorporation	80.92 ± 21.92

The equation (3) indicates that a meiofaunal individual of 1 nl in body mass ingests 3.51 times more volume of sediment than its own per hour. Such an enigmatic result was obtained because non-selective deposit feeding was supposed. In the deep sea, flux of particulate organic matter is limited, and most sedimentary particles are not valuable as food. In such environment, non-selective deposit feeding would be not a suitable feeding strategy, because the animals will waste energy by ingesting sedimentary particles that do not contain any nutrients.

According to the equation (4), 18,900 cells of bacteria per hour were ingested by a meiofauna of 1 nl in body volume. Taking the general size of the marine bacteria ($3.6 - 7.3 \times 10^{-8}$ nl : Lee and Fuhrman, 1987) into account, the volume of food ingested is reasonably small compared with the body size of meiofauna. This ingestion rate is equivalent to 0.378 ng of C/hr, assuming a bacterium cell contains 2×10^{-14} g of C (Lee and Fuhrman, 1987). This value is distinctively lower than those reported for shallow-water nematode species (6 – 17 ng of C/hr; Tietjen, 1980).

Assuming respiration of 1 nl O_2 is equivalent to the energy consumption of 0.4 ng of C (Heip *et al.*, 1985), an individual of deep-sea nematode and meiofauna other than nematodes (i.e. harpacticoid copepods, polychaetes and aplacophorans) of 1 nl in body volume will consume 0.304 and 0.159 ng of C/hr by respiration, respectively (Shirayama, 1992). Based on these values as well as ingestion rates obtained in the present study, budget of energy in deep-sea meiofauna seems to be just balanced. However, it should be noted that all the matter ingested will not necessarily be assimilated by the organisms. Tietjen (1980) reviewed that assimilation efficiency of ingested bacteria is 18.3 – 25.8 % in nematodes, and even lower for algae (Tietjen *et al.*, 1970).

It also should be noted that the ingestion rate estimated here is the maximal one, and several factors can be pointed out as a reason for overestimation. It is impossible for organisms to perfectly discriminate bacteria from inorganic sedimentary particle. A part of the label of meiofauna thus should come from ingesting inorganic particles that absorbed ^{3}H-thymidine through physico-chemical processes.

Another possible reason for overestimation is direct absorption of dissolved radioactive tracer by the meiofauna. Meiofauna may take up ^{3}H-thymidine dissolved in seawater by means of active absorption through its body surface, though Shirayama (1991) argued it is less likely. Meiofauna also might actively drink interstitial water which contained radioactive tracer (Lopez *et al.*,

1979), though Carman (1990 a) reported negative results using an autoradiography technique that uptake of ^{14}C-acetate by shallow-water copepods was entirely due to activity by epibiotic bacteria, and no radioactivity was detected inside of copepods. Some workers tried to overcome the problem related to the direct uptake of dissolved tracer by meiofauna using food items labelled separately before the ingestion experiment (Epstein and Shiaris, 1992; Taylor and Sullivan, 1984). This method however is not possible to use in the study of deep-sea meiofauna, because it is difficult to prepare the labelled deep-sea bacteria before the collection of deep-sea meiofauna.

Carman *et al.* (1989) showed that disturbance of sediment integrity alters grazing rates of benthic copepods significantly. If one plans to do experiments using the deep-sea sediment on board the ship or in the laboratory on land, it is not possible to carry out it without any disturbing of the sediment. Decompression also may affect on physiology of either meiofauna or microbes. Taking these potential artifacts into account, it is desired to develop a novel method that can be carried out *in situ* at depth in the future study regarding energy uptake of deep-sea meiofauna.

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