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Potential carbon sources for the head-down deposit-feeding polychaete *Heteromastus filiformis*

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ABSTRACT

In this study we investigated potential carbon sources for the capitellid polychaete, *Heteromastus filiformis.* It is a head-down deposit feeder ingesting sediment from at least 15 cm below the sediment-water interface. This orientation appears to minimize the worm's ability to acquire food and oxygen and maximize its exposure to sulfide. The food sources we examined were metabolically active bacteria, benthic algae, detritus and chemoautotrophic bacteria. Carbon retention efficiencies from metabolically active bacteria, benthic algae and detritus by *H. filiformis* were 26%, 8% and 4% respectively. These values are relatively low compared to other deposit feeding species suggesting that *H. filiformis* does not possess unique digestive capabilities. Rubisco (Ribulose bisphosphate carboxylase) assays were negative, which indicates an absence of symbiotic chemoautotrophic bacteria in tissue or absorbed carbon. Average $\delta\ ^{13}C$ were -12.83 for worms and -20.70 for 15 cm sediment, which indicates that external gardening of chemoautotrophs is not a major carbon source for *H. filiformis.* Nevertheless, several experiments showed that this capitellid worm had an unusually high gross heterotrophic CO_2 uptake. We suggest that *H. filiformis* utilizes both dissolved and particulate carbon sources stored within anoxic and sulfidic sediments that are not utilized by other deposit feeding organisms.

1. Introduction

Heteromastus filiformis (Claparede) is a head-down deposit-feeding polychaete which ingests sediment from at least 15 cm below the sediment-water interface (Cadee, 1979; Aller and Yingst, 1985). A head-down feeding orientation in near-shore marine sedimentary environments appears to minimize an organism's exposure to organic carbon which has been newly synthesized or deposited at the sediment surface. It also minimizes the worm's proximity to overlying oxygenated waters. Finally this position seems to maximize exposure to sulfide produced during sulfate reduction.

Diagenetic theory predicts that little of the surface-derived labile carbon will be present at depth in a sediment (Berner, 1980; but see Johnson, 1974, 1977; Kemp, 1990 and Lee, 1992). Rice and Rhoads (1989) presented a model showing that

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virtually none of the particulate carbon which supports growth in surface deposit feeders exists at the depth where *Heteromastus filiformis* feeds. They conclude that deep deposit feeders must either actively manipulate their immediate environment or else utilize food sources other than surface deposited particulate organic carbon if the assumptions of the diagenetic model are correct. The head-down deposit-feeding polychaetes *Scoloplos* spp. and *Leitoscolopolos fragilis* (Rice, 1986; Bianchi, 1988) are essentially feeding on surface material due to biologically or physically-mediated subduction at certain sites. Biologically enhanced subduction to depths of 15 cm on a yearly basis requires densities of >6000 adult *H. filiformis* per m^2 (Cadee, 1979). Densities at our field site are approximately 200 m^{-2}. Thus the carbon sources which *H. filiformis* utilizes may differ from those used by surface deposit feeders. Potential carbon sources available at depth include bacteria, detritus and dissolved organic compounds (Lopez and Levinton, 1987 and references therein).

In addition to potential organic carbon limitation, deep deposit feeders may be oxygen limited. Typically, only the top few mm of sediment in nearshore environments are oxic (Berner, 1980), so that many head-down deposit feeders, including *Heteromastus filiformis,* live within an anoxic environment. While *H. filiformis* can endure exposure to very low concentrations of oxygen for a few days, it requires oxygen for survival (Clough and Lopez, unpub.). Forbes and Lopez (1990) showed that low oxygen tensions limit the growth of *Capitella* sp. I, one of the smaller capitellids. *H. filiformis* must maintain contact with oxic overlying water to obtain oxygen.

Problems of potential food and oxygen limitation are exacerbated by high concentrations (up to mM) of H_2S within deeper sediments (Morse *et al.,* 1987; Somero *et al.,* 1989). Hydrogen sulfide is toxic to aerobic organisms (NRC, 1979), by virtue of its binding to hemoglobin and cytochrome c oxidase (Lehninger, 1982; Somero *et al.,* 1989). An aerobic organism must switch to less energetically favorable anaerobic pathways when exposed to toxic concentrations of H_2S, unless it is insensitive to sulfide (Powell, 1989). Sulfide may be beneficial to some *Capitella* spp. (Cuomo, 1985; Tsutsumi *et al.,* 1990; but see Dubilier, 1988).

Association with chemoautotrophic bacteria need not be symbiotic to be beneficial. External gardening of autotrophic bacteria by macrofauna has been proposed for deposit feeding organisms (Hylleberg, 1975; Aller, 1982; Jumars *et al.,* 1990). If such bacteria are an important carbon source, macrofauna feeding on them would be expected to have depleted $\delta\ ^{13}C$ signals but would not exhibit enzymatic activity unique to autotrophs.

Thus it appears that the life position of *Heteromastus filiformis* offers no benefits for acquiring organic carbon and oxygen, and avoiding sulfide. These geochemical variables are not independent within a sedimentary environment. One potentially beneficial interaction is association of the worm with chemoautotrophic bacteria (Jumars *et al.,* 1990; Tsutsumi *et al.,* 1990). The head-down orientation of *H. filiformis*

affords access to both subsurface sulfidic sediment and oxic overlying water. Several species of macrofauna containing chemoautolithotrophic symbionts have been found in environments containing oxygen and reduced sulfur (Fisher, 1990). Typically these animals lack a mouth and/or a gut. Several tests are used to determine the presence of bacterial symbionts, including Rubisco assays (ribulose bisphosphate carboxylase), δ^{13} C values, and measurements of CO_2 uptake (Fisher, 1990).

The purpose of this investigation was to determine the availability of sedimentary carbon sources for *Heteromastus filiformis.* Specifically we sought to determine (1) the availability of metabolically active bacteria, benthic algae and total organic carbon present in deep sediment to *H. filiformis;* (2) whether *H. filiformis* has chemoautotrophic symbionts and (3) if external chemoautotrophic bacteria are a primary food source for *H. filiformis.*

2. Materials and methods

a. Experimental animals

Heteromastus filiformis were collected from an intertidal muddy sand flat adjacent to an eelgrass bed in Great South Bay, Long Island, New York. The site is within Smith Point County Park. Densities of *H. filiformis* at this site are $\sim 200\ m^{-2}$. When collecting such a fragile polychaete it is impossible to retrieve whole individuals by sieving or picking. Instead we "panned" worms from sediment submersed in seawater, allowing collection of large numbers of unbroken individuals. Worms were then taken back to the lab where adhering sediment was removed. Worms used in CO_2 uptake experiments were collected one week, two days and immediately prior to beginning an experiment. In all other experiments worms were collected on the day before experiments were performed.

Nereis succinea (Frey and Leuckart) were collected from a mudflat adjacent to a saltmarsh in Flax Pond, Long Island, New York. Individuals were hand removed from the sediment and transported back to the lab where they were cleaned. *N. succinea* was collected two days or immediately prior to beginning an experiment.

b. Retention efficiency of particulate carbon

The purpose of these experiments was to determine absorption efficiency of organic carbon by *Heteromastus filiformis.* The dual tracer method (Calow and Fletcher, 1972; Lopez *et al.,* 1989) was used to evaluate absorption of bacteria, algae and total organic carbon. Sediments were labelled with both ^{14}C and ^{51}Cr. ^{51}Cr acts as an inert tracer while different ^{14}C compounds were used to label carbon sources (Lopez *et al.,* 1989; Lopez, 1993).

All experiments used the <63 μm fraction of field-collected sediment since *Heteromastus filiformis* feeds selectively on the silt clay fraction (Cadee, 1979). Ten μCi ^{51}Cr (NEN, 5 mCi $*$ ml^{-1}) was added to 10 ml of the sieved sediment for a final

activity of 1 μCi * ml^{-1} sediment. Fluorescent particles (Radiant Color, Richmond, Ca.) were added to 1 ml of sieved sediment to provide a visual marker of radioactive sediment. All treatments were incubated with ^{51}Cr for 12 hours, followed by a series of three rinses and centrifugations with 0.22 μm filtered seawater to remove non-incorporated label from the sediment.

i. Bacterial labelling. Sediment for this experiment was collected from > 15 cm depth one day prior to beginning the experiment, sieved, bubbled with N_2 gas for 30 minutes, sealed and allowed to incubate overnight at room temperature. Following ^{51}Cr incubation, 10 μCi ^{14}C mixed amino acids (NEN, 0.1 mCi * ml^{-1}) were added to the sediment. Incubation then proceeded for another 6 hours.

ii. Algal labelling. Benthic microalgae were labelled by adding 10 μCi ^{14}C bicarbonate (ICN, 10 μCi * ml^{-1}) to 5 ml freshly collected surface sediment (< 2 cm depth). ^{14}C and ^{51}Cr were added at the same time. Sediment was incubated in the light for 12 hours. We used surface sediment in these experiments because almost no living benthic algae are found at > 15 cm depth at our field site.

iii. Sedimentary organic matter labelling. ^{14}C formaldehyde was used to label organic matter in > 15 cm sediment (Lopez and Crenshaw, 1982; Lopez *et al.*, 1989). Sediment had been collected approximately one month prior to use, sieved, and frozen. Sediment was defrosted overnight and 25 μCi ^{14}C formaldehyde (NEN, 5 μCi * ml^{-1}) was added with ^{51}Cr to 5 ml sediment in a 30% NaCl solution. After a 12 hour incubation sediments were centrifuged and rinsed with filtered seawater five times to remove non-sorbed ^{14}C and ^{51}Cr.

iv. Feeding protocol. Heteromastus filiformis were collected the day before an experiment was begun. All worms had empty guts at the beginning of an experiment. Each worm was placed in a shallow culture dish with ~2 ml of > 1 mm non-labelled sand, which is too large for this species to ingest, and ~5 ml of filtered, aerated seawater. Fluorescent, radiolabelled sediment (~0.5 ml) was then added to each dish. Experiments were performed at room temperature (20°C).

Worms were observed microscopically for ingested sediment in the gut. It was not always possible to see ingested sediment through the body wall in the anterior segments, but pelletized sediment could be seen from that point on. Upon noting the ingestion of fluorescently-marked sediment, the first few individuals were collected prior to defecation and immediately frozen for later ^{14}C and ^{51}Cr analysis. Remaining worms were kept in the dishes for up to 20 hours. Fecal pellets were collected hourly. Average gut residence time in *Heteromastus filiformis* is about one hour (pers. obs.). Approximately half of the worms in any experiment fed. Absence of ingestion was confirmed by direct microscopic observation of gut contents during the experiment,

absence of fecal pellets within the sediment during and at the end of an experiment and background ^{51}Cr activity within worms at the end of an experiment.

Periodic water samples were taken from control dishes (no worms) to determine release of dissolved ^{14}C from sediments. Two 20 μl samples were used to determine total dissolved ^{14}C (TDC). Two additional samples were acid volatilized with 10% (w/w) TCA to remove radiolabelled DIC. Remaining ^{14}C was considered to be DOC. DIC was estimated from TDC-DOC.

Worms were collected, rinsed, and placed in 10% TCA (w/w) without allowing them to void gut contents. They were left in TCA overnight. A correction factor was applied to the few worms that contained sediment within their guts at the end of an experiment. After ^{51}Cr activity was determined in these (and all) worms, we multiplied ^{51}Cr dpm by the ^{14}C:^{51}Cr of the sediment and subtracted this amount prior to making any calculations. ^{51}Cr activity was determined on a LKB Compugamma gamma counter and ^{14}C activity was determined by liquid scintillation (LKB Rackbeta). The external standards method was used to estimate dpm.

It was not possible to employ the ratio method to calculate absorption efficiencies due to high uptake of dissolved ^{14}C during the algae and amino acid labelling experiments and high adsorption of ^{14}C formaldehyde to worms during the organic matter labeling experiments (see Results). Instead we used a mass balance approach to obtain ^{14}C retention as follows:

$$\text{C retention} = \frac{\text{CI} - \text{CD}}{\text{CI} + \text{CP}}$$

where:

$$\text{CI} = {}^{14}\text{C (dpm)} * \text{worm}^{-1} \text{ that ingested sediment}$$

$$\text{CD} = \text{mean } {}^{14}\text{C (dpm) of nonfeeding worms}$$

$$\text{CP} = \text{total } {}^{14}\text{C (dpm) of egested pellets}$$

Briefly, an individual *Heteromastus filiformis* which had ingested sediment had two associated ^{14}C measurements, CI and CP. The mean ^{14}C for all individuals which had not ingested sediment was also calculated (CD). CD was then subtracted from CI to correct for non-particulate uptake. A mass balance approach was then used to obtain carbon retention.

c. Chemoautotrophic investigation

i. Carbon dioxide uptake experiments. The purpose of these experiments was to determine the gross amount of CO_2 uptake by *Heteromastus filiformis.* Uptake of CO_2 during incubations using whole worms is due to both heterotrophic carbon replacement and, if symbionts are present, autotrophic carbon fixation. While these processes cannot be differentiated during short-term radiotracer experiments, one can

predict that if *H. filiformis* contains autotrophic symbionts it will exhibit elevated levels of CO_2 uptake. The dates on which the different CO_2 uptake experiments were performed are listed in Table 1.

Formalin-killed animals were run as controls in all experiments. The protocol for determining CO_2 uptake was adapted from Cavanaugh (1985). Each sample consisted of 10 ml 0.22 μm filtered seawater (pH adjusted to 9.0 with 0.5 M NaOH) in a 20 ml scintillation vial. One μCi ^{14}C sodium bicarbonate (ICN, 0.1 μCi $*$ ml^{-1}) was added to each vial. Experiments were begun by adding one worm to each vial. Samples were incubated in the dark for 8 hours. All experiments were done at room temperature (~ 20°). Worms were then collected, triple rinsed, blotted dry, frozen in liquid nitrogen and ground in 10% (w/w) TCA. Worm homogenates were bubbled with air for 20 minutes to remove acid volatile ^{14}C. After addition of scintillation cocktail, ^{14}C activity was measured by liquid scintillaton (LKB Rackbeta). Carbonate alkalinity was extrapolated from pH, salinity and temperature (Parsons *et al.,* 1984). CO_2 uptake rates were calculated as follows (Parsons *et al.,* 1984):

$$U = \frac{(RS - RB)*W}{R * N}$$

Where:

U = mgC $*$ $worm^{-1}*hr^{-1}$
RS = sample dpm
RB = blank dpm
W = weight of bicarbonate (mg)
R = added dpm
N = duration of experiment (hr).

The mean value of the 3 formalin-killed control worms that were used in every experiment was designated RB in all calculations to account for background activity and passive adsorption of radiolabel onto individual worms.

Effect of species type and gut fullness: CO_2 Experiments 1, 2, 3, and 4. The purpose of these experiments was to determine if *Heteromastus filiformis* exhibited elevated rates of CO_2 uptake. We chose to compare *H. filiformis* with *Nereis succinea,* a surface deposit feeding polychaete of similar size. We assumed that *N. succinea* did not contain symbionts and that CO_2 uptake was heterotrophic. Gross uptake rates in *N. succinea* were then used as a baseline to evaluate uptake rates in *H. filiformis.* Experiments 1 and 2 were run with *N. succinea* while experiments 3 and 4 used *H. filiformis.* Experiments were done on different dates (see Table 1).

In all experiments we also determined CO_2 uptake with and without sediment present in worm guts. We predicted that symbiont-driven CO_2 uptake may require

Table 1. Summary of CO_2 Uptake Experiments. Means (x) and standard deviations (sd) are given for each experimental group of size (n). Abbreviations for experimental groups are: N2M *Nereis succinea* starved for 2 days minus additional thiosulfate; NNM *N. succinea* not starved minus additional thiosulfate; H2M *Heteromastus filiformis* starved for 2 days minus additional thiosulfate; HMN *H. filiformis* not starved minus additional thiosulfate; HWM *H. filiformis* starved for one week minus additional thiosulfate; H2P *H. filiformis* starved for 2 days plus 0.1 mM thiosulfate; HNP *H. filiformis* not starved plus 0.1 mM thiosulfate; HWP *H. filiformis* starved for one week plus 0.1 mM thiosulfate.

Exp.	Date	Group	x (mgC * WORM^{-1} * HOUR^{-1})	(n)	sd
1	11/28/88	N2M	$1.31 * 10^{-6}$	6	$0.70 * 10^{-6}$
	11/28/88	NNM	$0.16 * 10^{-6}$	6	$0.27 * 10^{-6}$
2	1/07/89	N2M	$4.06 * 10^{-5}$	5	$1.40 * 10^{-5}$
	1/07/89	NNM	$2.96 * 10^{-5}$	6	$1.90 * 10^{-5}$
3	5/25/89	H2M	$7.08 * 10^{-5}$	12	$1.88 * 10^{-5}$
	5/25/89	HNM	$7.00 * 10^{-5}$	12	$1.41 * 10^{-5}$
4	5/31/89	H2M	$6.67 * 10^{-5}$	12	$2.81 * 10^{-5}$
	5/31/89	HNM	$8.33 * 10^{-5}$	12	$2.42 * 10^{-5}$
7	8/24/89	HWM	$0.88 * 10^{-5}$	12	$0.64 * 10^{-5}$
	8/24/89	H2M	$2.16 * 10^{-5}$	12	$1.29 * 10^{-5}$
	8/24/89	HNM	$2.03 * 10^{-5}$	12	$0.90 * 10^{-5}$
8	3/12/89	H2P	$2.55 * 10^{-4}$	8	$1.02 * 10^{-4}$
	3/12/89	H2M	$3.20 * 10^{-4}$	7	$0.78 * 10^{-4}$
	3/12/89	HNP	$2.70 * 10^{-4}$	8	$0.70 * 10^{-4}$
	3/12/89	HNM	$2.00 * 10^{-4}$	8	$0.80 * 10^{-4}$
9	10/23/89	HWP	$0.60 * 10^{-5}$	3	$0.10 * 10^{-5}$
	10/23/89	HWM	$0.73 * 10^{-5}$	3	$0.38 * 10^{-5}$
	10/23/89	H2P	$0.75 * 10^{-5}$	6	$0.34 * 10^{-5}$
	10/23/89	H2M	$0.58 * 10^{-5}$	6	$0.19 * 10^{-5}$
10	11/05/89	HWP	$0.24 * 10^{-5}$	5	$0.05 * 10^{-5}$
	11/05/89	H2P	$0.43 * 10^{-5}$	3	$0.15 * 10^{-5}$
	11/05/89	H2M	$0.47 * 10^{-5}$	5	$0.15 * 10^{-5}$
	11/05/89	HNP	$0.22 * 10^{-5}$	6	$0.08 * 10^{-5}$
	11/05/89	HNM	$0.18 * 10^{-5}$	5	$0.04 * 10^{-5}$
11	5/18/90	HWP	$0.93 * 10^{-4}$	6	$0.60 * 10^{-4}$
	5/18/90	HWM	$2.78 * 10^{-4}$	6	$1.19 * 10^{-4}$
	5/18/90	H2P	$2.35 * 10^{-4}$	6	$0.50 * 10^{-4}$
	5/18/90	H2M	$3.92 * 10^{-4}$	6	$2.59 * 10^{-4}$
	5/18/90	HNP	$1.42 * 10^{-4}$	6	$0.32 * 10^{-4}$
	5/18/90	HNM	$2.32 * 10^{-4}$	6	$0.84 * 10^{-4}$
12	8/08/90	HWP	$1.66 * 10^{-4}$	9	$0.85 * 10^{-4}$
	8/08/90	HWM	$2.12 * 10^{-4}$	9	$0.76 * 10^{-4}$
	8/08/90	H2P	$1.08 * 10^{-4}$	8	$0.43 * 10^{-4}$
	8/08/90	H2M	$1.09 * 10^{-4}$	8	$0.52 * 10^{-4}$

the presence of reduced sediment. Half the worms were collected 2 days prior to the beginning of an experiment. During this holding time worms evacuated all gut contents. The other worms were collected immediately prior to experiments and had full guts. Because sediment weight within the gut is large relative to worm wet weight, results will be given on a per worm basis to allow comparison between full and empty gut individuals. We used worms of approximately the same size (~ 10 mg wet weight) in all treatments.

Time course of CO_2 uptake: CO_2 Experiments 5 and 6. Experiments 5 and 6 determined the time course of CO_2 uptake by *Heteromastus filiformis.* As with Experiments 1–4 both freshly collected worms and worms which had been starved for 2 days were used in these experiments. Time zero in each experiment was addition of ^{14}C $NaHCO_3$ to vials which each contained one worm. At 1 to 2 hour intervals three individuals were sacrificed. The time specific uptake was calculated as the mean of the three individuals terminated at that time point.

Effect of starvation: CO_2 Experiment 7. The purpose of Experiment 7 was to determine the effects of prolonged starvation of *Heteromastus filiformis* on CO_2 uptake. We predicted that if a temporary store of reduced sulfur exists within *H. filiformis* then it should be exhausted during a 1 week starvation period. One third of the worms were collected one week prior to beginning the CO_2 uptake experiment, one third were collected two days prior to an experiment and the remaining worms were collected the day of the experiment.

Effect of thiosulfate addition: CO_2 Experiments 8, 9, 10, 11 and 12. These experiments determined whether addition of thiosulfate stimulated CO_2 uptake by *Heteromastus filiformis.* The outer sulfur atom in thiosulfate has the same oxidation state (−2) as sulfide and can serve as a reductant for chemoautotrophic bacteria (Kelly, 1982). Because there is no reason to suspect that heterotrophic uptake of CO_2 will increase with addition of thiosulfate enhanced uptake should be due to autotrophic carbon fixation alone (Cavanaugh, 1985). In these experiments half of the samples were incubated with seawater containing 0.1 mM NaS_2O_3.

In Experiment 8, worms were starved for two days or collected just prior to beginning the experiment. In Experiments 9 and 12 worms were starved for two days or one week. Experiments 10 and 11 used individuals which had been starved for two days, one week or collected just prior to the experiment. All experiments were done on different days.

ii. Rubisco assays. Worms were assayed for ribulose bisphosphate carboxylate (Rubisco), an enzyme unique to autotrophic organisms (Felbeck, 1981; Cavanaugh, 1985). Approximately 6 starved worms were pooled to obtain 50 mg (wet weight) tissue. Homogenates were prepared in 0.02M $MgCl_2$ buffer as described in Cavanaugh

(1985). Homogenates were then sonicated, centrifuged and the supernate was decanted and assayed.

0.5 μCi ^{14}C sodium bicarbonate (ICN, 10 μCi * ml^{-1}) was added to each sample. 40 μl 8.85 mM RuBP (Ribulose bisphosphate, Fisher Chem.) was added to half of the aliquots. Distilled water was added to the remaining aliquots and served as a control. The reaction was stopped at 1, 2, 5, 10 and 20 minute intervals by addition of 400 μl glacial acetic acid. Samples were then vortexed, bubbled with air and treated as previously described for the CO_2 uptake experiments.

Concurrent positive controls for Rubisco activity were run with spinach homogenate. Scintillation cocktail was added to samples and ^{14}C activity was determined as previously described.

iii. DEL ^{13}C. δ ^{13}C values were determined by the Light Stable Isotope Facility at Harvard University. All samples are reported relative to a PDB carbonate standard. Two subsamples of a group of 10 *Heteromastus filiformis* cleared of all sediment and dried at 30°C were measured. *H. filiformis* fecal pellets were collected from pellet piles at the field site and analyzed. In addition, sediment was collected from our field site at >15 cm depth, sieved to <63 μm and analyzed for stable carbon. All samples were collected in June 1991.

3. Results

a. Retention efficiency of particulate carbon

In all experiments worms that did not ingest sediment incorporated ^{14}C activity (Figs. 1a, 2a and 3a). This indicates that other pathways besides ingestion provided carbon to *Heteromastus filiformis* under the experimental conditions used. It also precludes use of dual tracer techniques to measure absorption efficiencies.

i. Retention of bacteria. Heteromastus filiformis retained an average of 26% of metabolically active bacteria (Fig. 1a). Non-feeding worms retained ~35% of the carbon which was retained from particulate sources by feeding worms. We do not know how much ^{14}C was respired as $^{14}CO_2$ in either group.

Water samples indicated that dissolved ^{14}C was present throughout the experiment (Fig. 1b). Respiration and recycling of radiolabelled compounds was certainly present in this experiment. Acid-volatilization indicated that the dissolved ^{14}C was predominantly CO_2. No temporal patterns of CO_2 release were detected.

ii. Retention of algae. The mean retention of algal carbon by feeding *Heteromastus filiformis* was 8% (Fig. 2a). In this experiment the mean ^{14}C retained by non-feeding worms was actually slightly greater than that which was provided by ingestion of particulate carbon.

The patterns of dissolved ^{14}C released by the sediment shown in Figure 2b are very

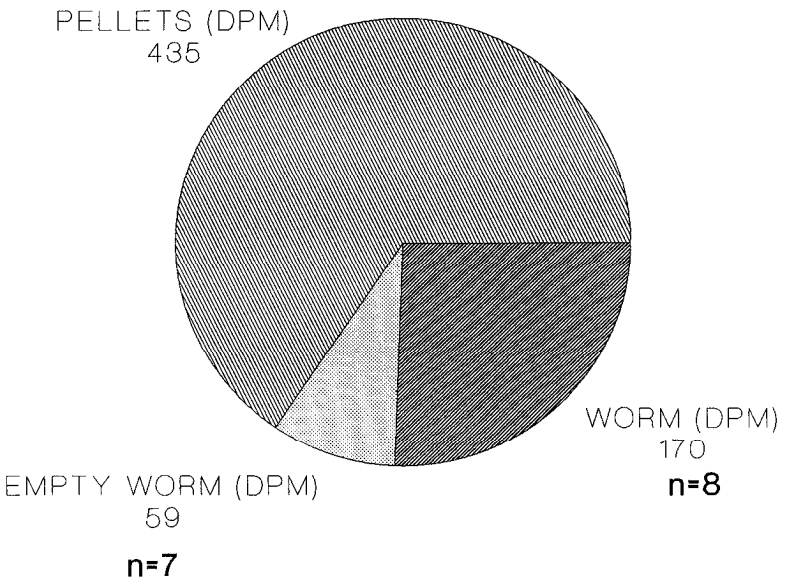

Figure 1. Bacterial retention by *Heteromastus filiformis.* (a) DPM activities in worms and pellets. Numbers are averages of n replicates. CI = Worm + Empty Worm. CP = Pellets. CD = Empty Worm. See text for equation. (b) Dissolved ^{14}C activity (CO_2 and non acid-volatile) in overlying water during the course of the bacterial retention experiment.

similar to those shown in Figure 1b for bacteria. There was always $^{14}CO_2$ in the overlying water.

iii. Retention of sedimentary organic carbon. The mean retention efficiency for sediment organic matter labelled by the formaldehyde technique was 4% (Fig. 3a). The mean dpm of non-feeding worms was much higher than ^{14}C provided by sediment ingestion alone.

The dissolved ^{14}C showed very different patterns than those exhibited during bacterial and algal labelling experiments (Fig. 3b). First, ^{14}C present in overlying water was an order of magnitude lower than in the previous experiments. Second, there was no indication that the dissolved ^{14}C was predominantly acid-volatile. Clearly, dissolved ^{14}C release from ^{14}C-formaldehyde labeled sediment differs from release from labeled bacteria and algae.

b. Chemoautotrophic investigation

i. Carbon dioxide uptake experiments

Effect of species type and gut fullness: CO_2 Experiments 1, 2, 3 and 4. Mean CO_2 uptake by *Heteromastus filiformis* varied between $7.0 * 10^{-5}$ mg C * worm^{-1} * hr^{-1} and $8.33 * 10^{-5}$ mg C * worm^{-1} * hr^{-1} (Table 1). Mean uptake rates by *Nereis succinea* varied from $0.16 * 10^{-6}$ to $4.06 * 10^{-5}$ mg C * worm^{-1} * hr^{-1}. Nonparametric Wilcoxon

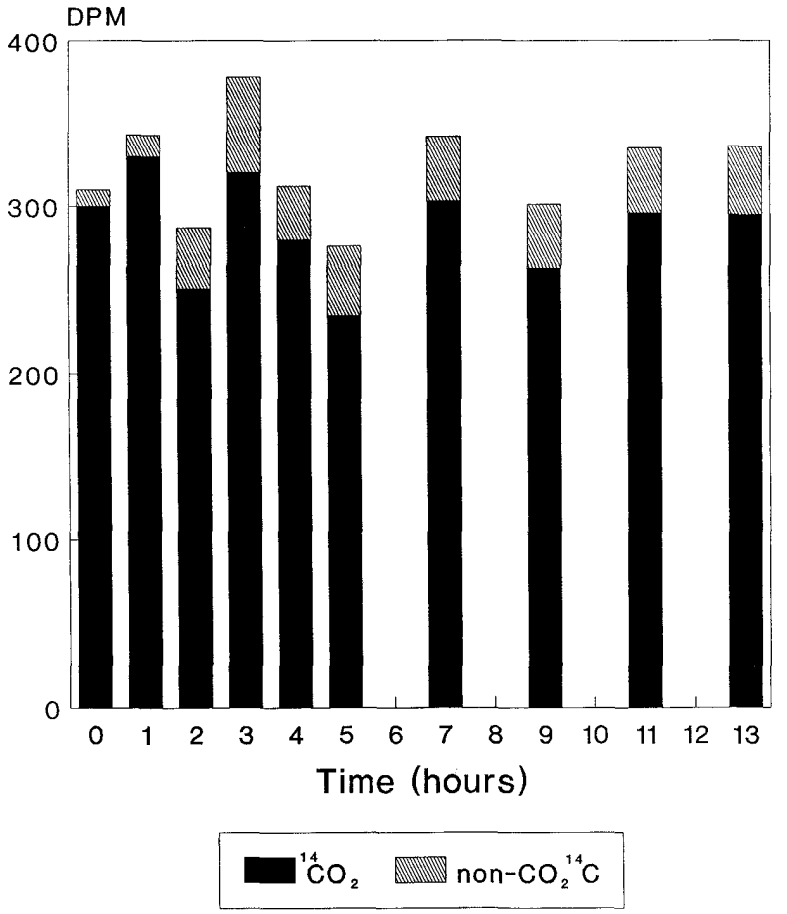

Figure 1. (Continued)

multiple comparisons showed that in all but one comparison, CO_2 uptake by *H. filiformis* was either significantly or nearly significantly greater than uptake by *N. succinea* (Fig. 4). The presence of sediment within a *H. filiformis* gut did not affect CO_2 uptake.

Time course of CO_2 uptake: CO_2 Experiments 5 and 6. Mean values for CO_2 uptake were $2.92 * 10^{-4}$ mg C * worm^{-1} * hr^{-1} for starved worms and $2.52 * 10^{-5}$ mg C * worm^{-1} * hr^{-1} for full worms in experiment 5 and $6.64 * 10^{-5}$ mg C * worm^{-1} * hr^{-1} and $4.14 * 10^{-5}$ mg C * worm^{-1} * hr^{-1} for starved and full worms during experiment 6. To provide uptake rates on a mg C * mg^{-1} wet weight of worm we only present data for starved worms in Figure 5. In experiment 5 ^{14}C uptake increased consistently with time (Fig. 5). There was no consistent pattern of CO_2 uptake in experiment 6 (Fig. 5). Experiment 5 and 6 were performed one week apart in July 1989.

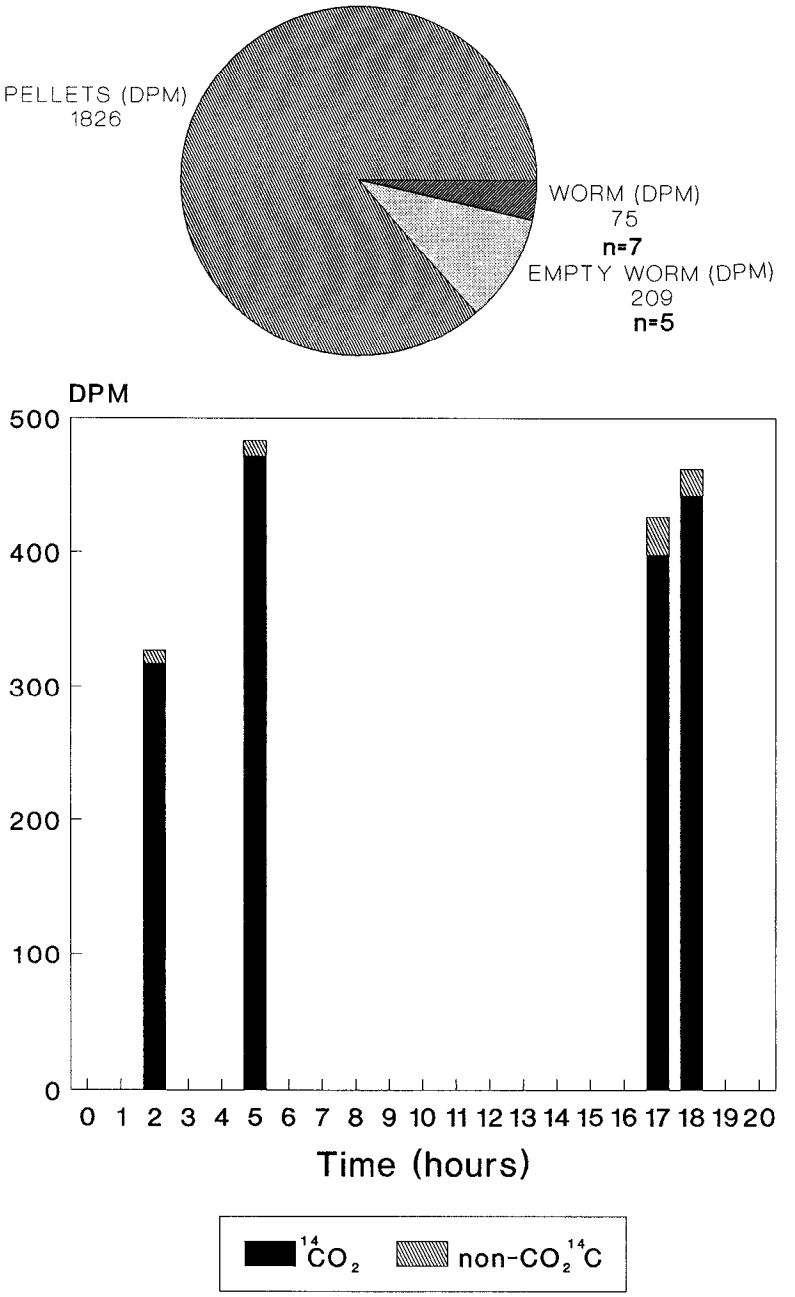

Figure 2. Algal carbon retention by *Heteromastus filiformis.* (a) DPM activities in worms and pellets. Numbers are averages of n replicates. CI = Worm + Empty Worm. CP = Pellets. CD = Empty Worm. See text for equation. (b) Dissolved ^{14}C activity (CO_2 and non acid-volatile) in overlying water during the course of the algal retention experiment.

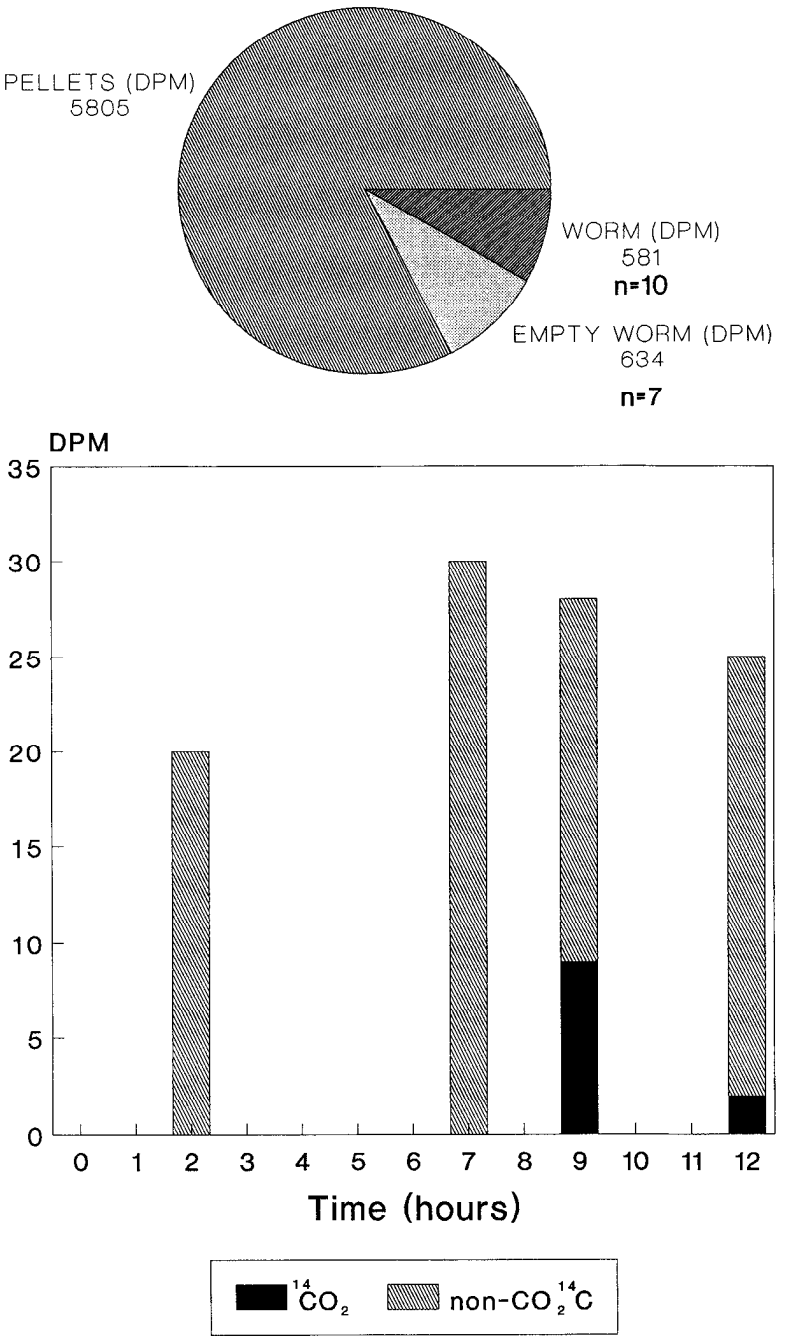

Figure 3. Detrital carbon retention by *Heteromastus filiformis*. (a) DPM activities in worms and pellets. Numbers are averages of n replicates. CI = Worm + Empty Worm. CP = Pellets. CD = Empty Worm. See text for equation. (b) Dissolved ^{14}C activity (CO_2 and non acid-volatile) in overlying water during the course of the detrital carbon retention experiment.

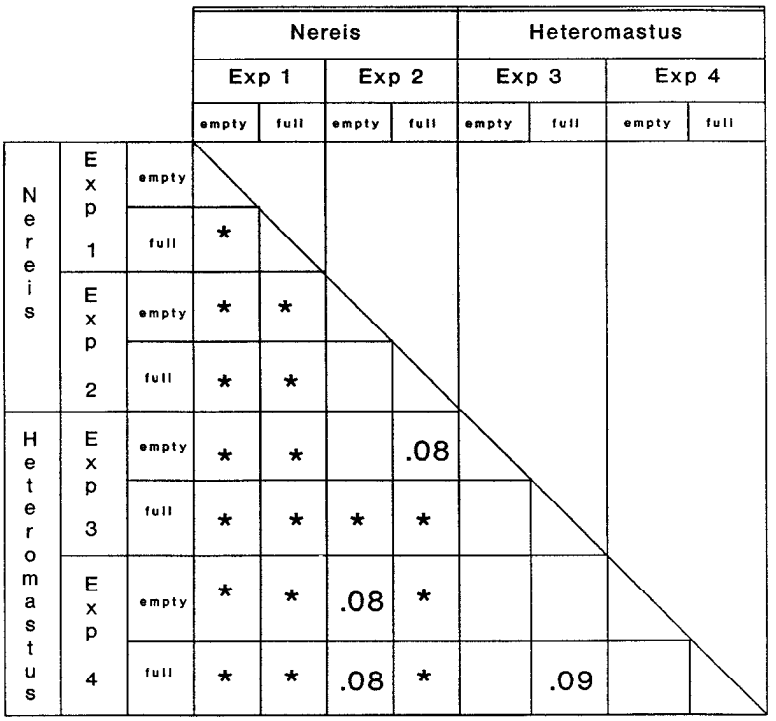

Figure 4. CO_2 uptake experiments 1–4. Results of a nonparametric Wilcoxon multiple comparisons test for effect of species type and gut fullness on CO_2 uptake. Means and standard deviations are given in Table 1.

Effect of starvation: CO_2 Experiment 7. Mean CO_2 uptake rates were $0.88 * 10^{-5}$ mg C * worm^{-1} * hr^{-1}, $2.16 * 10^{-5}$ mg C * worm^{-1} * hr^{-1}, and $2.03 * 10^{-5}$ mg C * worm^{-1} * hr^{-1} for worms starved for one week, two days and freshly collected respectively (Table 1). There was no effect of increased length of starvation period.

Effect of thiosulfate addition: CO_2 Experiments 8, 9, 10, 11 and 12. The highest mean CO_2 uptake occurred in Experiment 11 with worms that had been starved for 2 days without added thiosulfate, while the lowest mean was in Experiment 11 for worms that had not been starved or exposed to added thiosulfate (Table 1). A nonparametric Wilcoxon multiple comparisons test showed that thiosulfate addition did not affect CO_2 uptake. There was unexpected variability between experiments (Fig. 6). Dates of the experiments are listed in Table 1.

ii. Rubisco assays

There was no detectable Rubisco activity within *Heteromastus filiformis* (Fig. 7). Positive controls using spinach extract showed Rubisco activity.

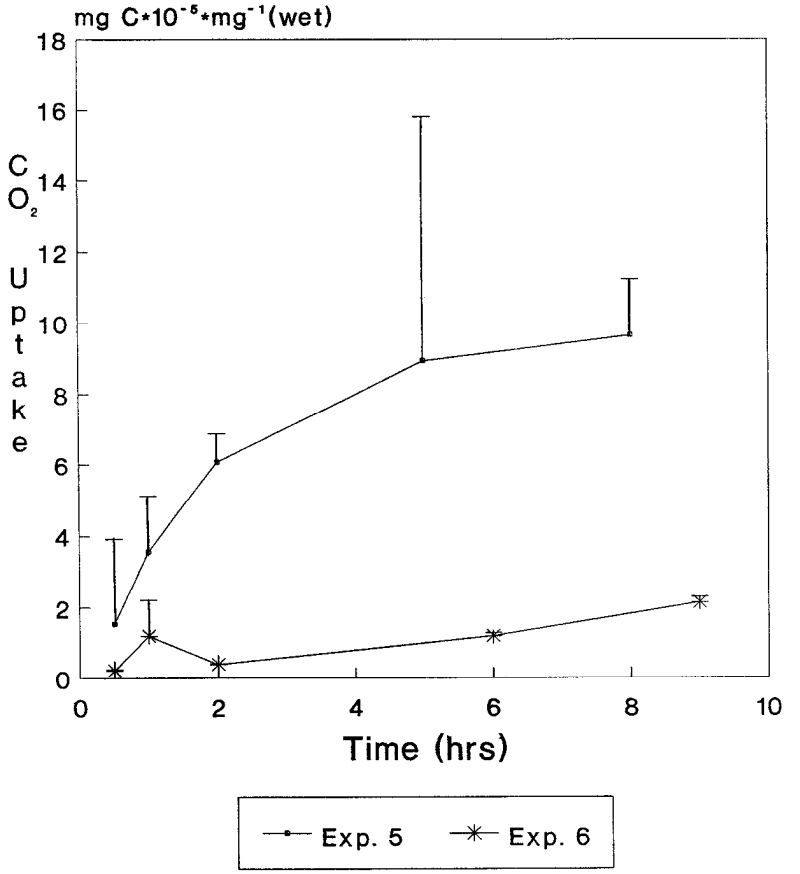

Figure 5. CO_2 uptake experiments 5–6. Time course of CO_2 uptake. Values are the mean of three worms per time point. Only data for starved worms are graphed. Error bars represent plus one standard deviation.

iii. DEL ^{13}C

Heteromastus filiformis were surprisingly heavy with a δ ^{13}C of -12.83. In contrast fecal pellets and sediment were similar at -18.07 and -20.70, respectively.

4. Discussion

Deep deposit feeders obtain carbon from sediments that are assumed to be depleted in labile carbon relative to surface sediments. It is not obvious what food sources are utilized by these organisms. Several mechanisms have been proposed to explain how this functional group makes a living including enhanced geophysical mixing (Rhoads, 1974; Bianchi, 1988; Bianchi and Rice, 1988; Rice and Rhoads, 1989), external gardening of bacteria (Hylleberg, 1975; Aller, 1982; Aller and Yingst,

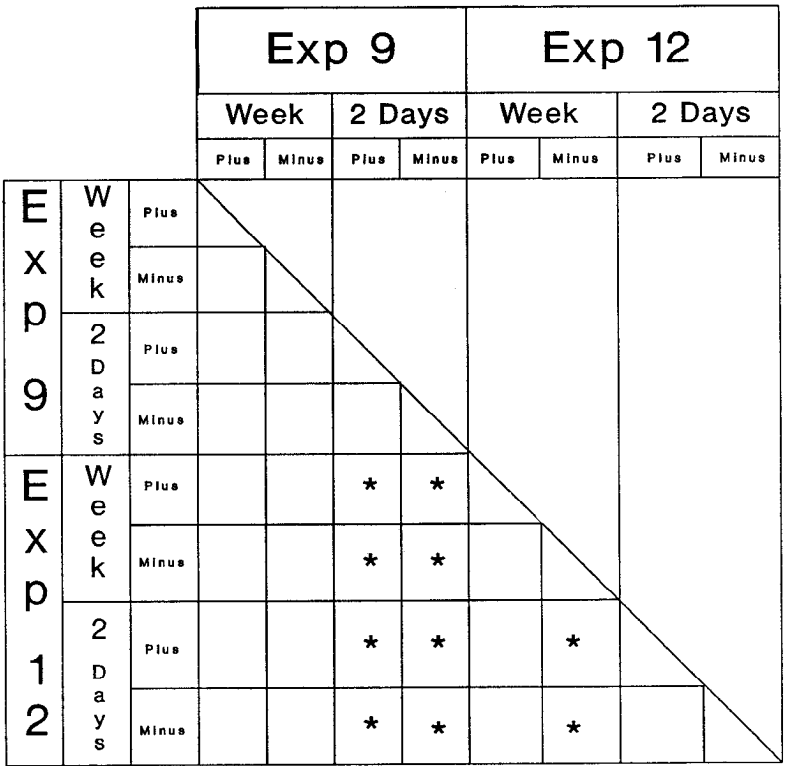

Figure 6. CO_2 uptake experiments 9 and 12. Results of a nonparametric Wilcoxon multiple comparisons test for effects of addition of 0.1 mM thiosulfate on CO_2 uptake by *Heteromastus filiformis.* Means and standard deviations are given in Table 1.

1985), caching (Jumars *et al.,* 1990) and association with chemoautotrophic symbionts (Jumars *et al.,* 1990; Tsutsumi *et al.,* 1990). Previous studies have not directly evaluated the uptake of carbon by head-down deposit feeders. We investigated several potential carbon sources for the head-down deposit feeding polychaete *Heteromastus filiformis.*

Retention efficiencies of algal, bacterial and detrital carbon were low in comparison to those measured for surface deposit feeders (Cammen, 1980; Lopez and Levinton, 1987; Lopez *et al.,* 1989; but see Kemp, 1986). For example, *Nereis succinea* absorbed 57% of the carbon in metabolically active bacteria (Cammen, 1980) while *Heteromastus filiformis* retained 26%. It does not appear that *H. filiformis* has unique digestive abilities to use particulate carbon sources. In fact it seems to be less efficient than other deposit feeders at extracting carbon from algae, bacteria and detritus.

We did not expect to find high ^{14}C activity in non-feeding worms in the retention efficiency experiments. We conclude from the dissolved ^{14}C measurements that this

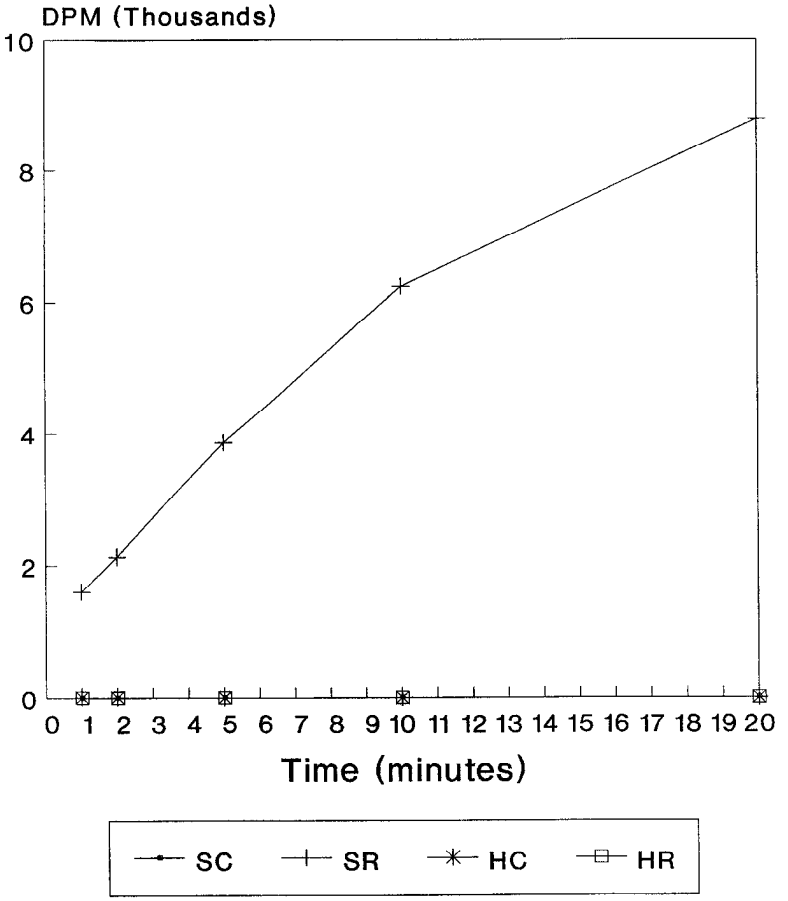

Figure 7. Rubisco experiment. Time course of Rubisco activity in homogenates of *Heteromastus filiformis* and spinach. SC: Spinach control (No RUBP added); SR: Spinach reactive (RUBP added); HC: *Heteromastus* control (No RUBP added); HR: *Heteromastus* reactive (RUBP added).

activity was due to the uptake of $^{14}CO_2$ released by algae and bacteria. The occurrence of CO_2 uptake in *Heteromastus filiformis* is supported by our direct measurements of radiolabelled CO_2 uptake (CO_2 Experiments 1–12). The $^{14}CO_2$ taken up by worms must have been incorporated into non-acid volatile compounds because worms were dissolved in 10% TCA prior to scintillation counting. Unincorporated CO_2 would be driven off with this acidic treatment.

We offer a different explanation for ^{14}C uptake by non-feeding worms in the sedimentary organic matter retention experiment. Little DIC or DOC was present in the overlying water. This supports the conclusion of Lopez and Crenshaw (1982) that ^{14}C formaldehyde is not easily metabolized by bacteria once it is adsorbed to organic

matter. Instead we suspect that high ^{14}C activity was due to desorption of sediment-bound ^{14}C and subsequent adsorption to the worms. One *Heteromastus filiformis* represents a large amount of organic matter compared to sedimentary organic carbon, thus introducing an ideal surface for formaldehyde adsorption. This should not be a problem in pulse-chase feeding experiments (Lopez and Crenshaw, 1982) but should be noted for other types of experiments employing this labelling technique.

CO_2 uptake experiments showed that CO_2 uptake by *Heteromastus filiformis* was consistently higher than uptake by *Nereis succinea,* although there was significant intraspecific variability between experiments in both species. Rates did not vary with thiosulfate addition nor were they dependent on the presence of reduced sediment in gut contents.

We initially hypothesized that enhanced uptake of CO_2 by *Heteromastus filiformis* would be indicative of chemoautotrophic activity. This is not the case. Rubisco activity has been found in all organisms containing chemoautotrophic symbionts (Fisher, 1990). There was no Rubisco activity in *Heteromastus,* demonstrating that it does not harbor chemoautotrophic symbionts. Therefore, enhanced CO_2 uptake must be heterotrophic. One explanation is that *H. filiformis* has a higher rate of anapleurotic exchange reactions than other polychaetes, possibly due to heme production (Lehninger, 1982; Hochachka and Somero, 1984). While numerous carboxylation reactions are known (Lehninger, 1982) we can not propose a mechanism for elevated rates of CO_2 uptake without further investigations.

$\delta\ ^{13}C$ results indicated that *Heteromastus filiformis* is enriched in ^{13}C relative to surrounding sediments. Similar enriched values have been found in *Capitella* spp. (Spies *et al.,* 1989). If *H. filiformis* were using chemoautotrophs as a carbon source it should be relatively depleted in ^{13}C (Haines and Montgue, 1979; Rau, 1981). It is important to note that enriched $\delta\ ^{13}C$ values exclude both symbiotic autotrophs and external gardening of chemoautotrophs as important sources of anabolic carbon for *H. filiformis.* Major carbon sources for *H. filiformis* must be enriched in ^{13}C. Possible sources include eelgrass, a C-4 plant with a $\delta\ ^{13}C$ of -11 (Peterson *et al.,* 1986) and dissolved organic compounds in porewater (Blair *et al.*, 1987). In this study we suspect that eelgrass detritus was not important because the δ 13C of the sedimentary organic carbon did not reflect its heavy isotopic signal. The potential for uptake of dissolved compounds from porewater is not a new idea (Stephens, 1968; Jørgensen, 1976 and references therein) and results from preliminary experiments in our lab show that *H. filiformis* exhibited high acetate uptake rates (Clough and Lopez, unpub.). A final possibility is that enriched $\delta\ ^{13}C$ values reflect partial incorporation of CO_2 from overlying seawater ($\delta\ ^{13}C \sim 0$, Schmaljohann *et al.,* 1990) into the worms.

In summary the consistent association between *Heteromastus filiformis* and anoxic, sulfidic sediments is not explained by either bacterial symbionts or gardening of chemoautotrophic bacteria. Particulate feeding must provide carbon to this deep

deposit feeder. Typically this carbon has been assumed to be predominantly refractory. It has recently been proposed that increased carbon preservation associated with anoxic sediments is not due to intrinsically slower rates of anaerobic decomposition, or to the largely refractory nature of this carbon (Lee, 1992). Instead, the absence of bacterial grazers due to lack of oxygen and/or sulfide toxicity eliminates the sedimentary microbial loop and leads to sequestering of bacteria and bacterial products within anoxic sediments (also see Kemp, 1990). If this is true, then sulfide-tolerant organisms which can reach deep anoxic sediments while acquiring oxygen from overlying waters will gain access to a pool of labile carbon which is literally out of reach for other aerobic organisms.

Assuming that *Heteromastus filiformis* retains 4% of ingested sedimentary organic matter and has an average egestion rate of 4 body weights per day, we can make a rough carbon budget. If we assume a relatively high value for particulate carbon of 1.0% (dry wt.) for deep anoxic sediment (Rice and Rhoads, 1989), then a 1 mg (dry weight) worm would retain $1.6 * 10^{-3}$ mg C. If a worm is 40% C (dry weight) and turns over 1% body carbon daily for maintenance, a 1 mg worm would require $4 * 10^{-3}$ mg C per day.

This suggests that up to 50% of the carbon required by *Heteromastus filiformis* can be provided through deposit feeding on what has been classified as low quality food. Sediment at *H. filiformis* feeding depths can have much less than 1% organic carbon, so the above estimate is probably a maximum. It is perplexing that uptake of CO_2 within the worm seems to provide similar amounts of carbon. The possibility that uptake of dissolved components in surrounding porewater may also be essential to *H. filiformis* requires further investigation. We suggest that *H. filiformis* is utilizing both dissolved and particulate carbon sources within anoxic, sulfidic sediments that are inaccessible to most other aerobic organisms.

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