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Exploitation of a chemosynthetic food resource by the polychaete *Capitella* sp. I

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ABSTRACT: In organically enriched sediments of coastal areas, sulfate-reducing bacteria decompose organic matter anaerobically, producing high levels of hydrogen sulfide. Chemoautotrophic sulfur-oxidizing bacteria proliferate at the sulfide/oxygen interface and use hydrogen sulfide as an electron donor. A few species of small polychaete worms, including *Capitella* sp. I, often dominate the macrofaunal benthic communities in such sulfide-rich environments in the organically enriched sediments. In this study, we conducted 2 laboratory experiments to determine whether *Capitella* sp. I can benefit trophically through the exploitation of the organic matter chemosynthetically produced by sulfur-oxidizing bacteria. In the first experiment, we reared juveniles of *Capitella* sp. I with natural sediment of very low organic content, with no additional organic matter, under dark conditions, and exposed them to 3 different levels of sodium sulfide. The worms reared in the sulfide treatments showed better survival, enhanced growth and reproduction. They had lower $\delta^{13}C$ values (–24.3‰, mean) than control worms (–20.1‰, mean) and the sediments in which the worms were cultured (–21.4‰, mean). The distinctive $\delta^{13}C$ signature of the worms in the sulfide treatments indicates that they did not share the same carbon source as the control worms. The second experiment was done in the same manner as the first, but the carbonate in the water was replaced with $^{13}CO_2$ in order to trace the autotrophic fixation of carbon dioxide by chemosynthetic bacteria occurring within the sediments. The results indicate that fixation of $^{13}CO_2$ was promoted in sediments with Na_2S amendments, and further enhanced by the presence of *Capitella* sp. I. The worms in these sediments had extremely high $\delta^{13}C$ values (+5218.2‰). The results of this study introduce the possibility of enhanced survival and growth of *Capitella* sp. I in sulfide-rich environments in the organically enriched sediments, facilitated by its utilization of a novel source of organic matter.

KEY WORDS: *Capitella* · Chemoautotrophic bacteria · Stable isotope · Organic enrichment · ^{13}C · ^{15}N

INTRODUCTION

The species composition of macrobenthic communities throughout the world is strongly dependent upon the sedimentary organic content. In organically enriched sediments, macrobenthic communities tend to be dominated by only a few species of small annelids including the polychaetes, *Capitella* sp. I (Grassle & Grassle 1976), other members of the *Capitella* complex, and spionids, while more diverse communities that include molluscs, crustaceans and echinoderms may predominate in sediments that are less organically enriched (Reish 1971, 1979, Kitamori 1975, Pearson & Rosenberg 1976, 1978).

Previous studies on the association between *Capitella* species and organically enriched sediments were con-

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cerned with 'physiological tolerance to the low oxygen conditions' in organically enriched sediments (Reish 1970) and 'life-history adaptations' (Grassle & Grassle 1974). The reasons for *Capitella* spp.s' association with organically enriched sediments still are not entirely clear, although it has been shown experimentally that their larvae may choose organic-rich mud over sand at the time of settlement and metamorphosis (Butman & Grassle 1992, Grassle et al. 1992). Our laboratory experiments (Tsutsumi et al. 1990) and others (Tenore 1983, Alongi & Tenore 1985) have shown that growth of these *Capitella* species is independent of sedimentary organic content, but is correlated with the amount of labile organic matter.

Our field work on the population dynamics of *Capitella* sp. (Tsutsumi 1987, 1990) revealed that the populations occurring on the tidal flat, and below a fish culture net pen, were severely reduced during the summer, following the development of reducing conditions in the organically enriched sediments, but that tiny patches (with extremely low densities) remained in the less organically enriched sediments within the habitats. When the surface of the organically enriched sediments was oxidized, the remnant patches soon recolonized and exhibited a large potential for population growth. Large, dense patches of over 40 000 individuals m^{-2} were re-established within only 2 to 3 mo. This rapid growth process was reproduced in organically enriched sediments under experimental conditions (Bridges et al. 1994, Levin et al. 1996). These studies clarified the demographic processes associated with the rapid population growth (e.g. fast individual growth, early maturation, and increased fecundity per brood). Field work and laboratory studies seem to suggest that the *Capitella* species favor organically enriched sediments as a temporary habitat (in which a large amount of labile organic matter, essential to their rapid population growth, is available).

Over the past 2 decades, much has been learned about the abundant benthic communities around hydrothermal vents in the deep sea, where high levels of hydrogen sulfide support primary production by chemoautotrophic sulfur-oxidizing bacteria (Corliss et al. 1979, Rau 1981, Grassle 1985, Tunnicliffe et al. 1985, Hashimoto et al. 1993). Recently, a *Capitella* species and other capitelid polychaetes also have been found around shallow hydrothermal vents in sulfide-rich conditions in the Mediterranean Sea (Kamenev et al. 1993, Dando et al. 1995, Thiermann et al. 1997). The *Capitella* species, which is referred to as *Capitella* sp. M, possesses a similar life history to *Capitella* sp. I (e.g. short life cycle, production of free-swimming larvae), but exhibits a higher tolerance to hypoxia and high-sulfide conditions (Gamenick et al. 1998b). Gamenick et al. (1998a) measured high sulfide levels (approximately 300 to 1400 µM) in sediments inhabited by *Capitella* sp. M close to vent outlets. *Capitella* sp. M demonstrated a high tolerance for hydrogen sulfide (740 µM in anoxic conditions), suggesting the possibility that organic matter produced chemosynthetically by sulfur-oxidizing bacteria may be available for use by *Capitella* species.

Similar sulfide-rich environments exist in the organically enriched sediments of estuaries and on mud flats, where sulfate-reducing bacteria decompose organic matter anaerobically, releasing hydrogen sulfide as a byproduct (Jørgensen & Fenchel 1974, Jørgensen 1977a,b, Martens 1984). In these shallow-water environments, the presence of symbiotic chemoautotrophic bacteria able to exploit hydrogen sulfide as a source of energy has been reported in some benthic species, including bivalves, vestimentiferans, pogonophores, and oligochaetes (Felbeck et al. 1983, Cavanaugh 1985, Vetter 1985, Giere et al. 1988, Fenchel & Findlay 1989, Schmaljohann et al. 1990). Although *Capitella* species do not harbor endosymbiotic bacteria (Felbeck et al. 1981, Cuomo 1985), it is possible that they may benefit trophically from the additional supply of labile organic matter produced by chemosynthetic bacteria in organically enriched sediments.

Another question that has not been adequately addressed is how *Capitella* species maintain themselves in sediments of low organic content between periods when organically rich sediments become available. It is possible that *Capitella* spp. exist at extremely low densities in sediments of low organic content, living suboptimally, but nevertheless producing small numbers of larvae (Grassle & Grassle 1974, 1976, Tsutsumi & Kikuchi 1984, Tsutsumi 1990). In such sediments with low organic content, chemosynthetically produced organic matter may be available around the redox potential discontinuity layer, the interface between oxic and reduced conditions where sulfur-oxidizing bacteria may thrive (Nelson 1992).

In the present study, we conducted 2 laboratory experiments to determine whether *Capitella* sp. I can exploit chemosynthetically produced organic matter. In Expt 1, we reared juvenile *Capitella* sp. I in natural sediment of very low organic content, with no external supply of organic matter, and under dark conditions. The cultures were exposed to 3 different levels of AVS (acid-volatile sulfide). We measured the body sizes of the worms, determined carbon and nitrogen stable-isotope ratios of both the sediment and worms, and examined whether *Capitella* sp. I could grow and complete its life cycle. In Expt 2, we reared *Capitella* sp. I juveniles in the same manner as in Expt 1, but we enriched the carbonate in the water with ^{13}C. We determined carbon and nitrogen stable-isotope ratios of the sediment and worms and examined how much

inorganic ^{13}C in the water was incorporated into the organic matter of the sediment through chemosynthesis by sulfur-oxidizing bacteria and then assimilated by the worms through their feeding on the sediment.

The goal of this study was to clarify whether *Capitella* sp. I growing in the presence of sulfide can exploit chemosynthetically produced organic matter as one of the sources of available organic matter.

MATERIALS AND METHODS

The culture of *Capitella* sp. I (Grassle & Grassle 1976) maintained in our laboratory was originally established using worms collected from Tomoe Cove, Amakusa, Japan. Identity was confirmed by cross-breeding with known *Capitella* sp. I (J. P. Grassle pers. comm.). Mud for use in the laboratory experiments was collected from Hiketa Bay, Seto Inland Sea, Japan (silt-clay content = 84.4 %, total organic carbon and nitrogen contents = 2.06 and 0.21 %, respectively). The mud was frozen at –20°C, thawed, and homogenized with a spatula (stirring for 1 min) before experiments. Sea water was filtered with membrane filters (Advantec, cellulose nitrate 0.45 µm) and glass-fiber filters (Advantec, GC50). Plastic containers (20 × 30 × 10 cm) were filled with 1.5 kg of mud (sediment depth = 2 cm) and 2 l of filtered sea water.

Expt 1 (Table 1). We collected 300 brood tubes of *Capitella* sp. I from the laboratory culture and isolated them individually in petri dishes with sea water. We collected daily the free-swimming larvae that hatched out from the brood tubes and kept the larvae in a flask with sea water. Six experimental containers were prepared. To each of them, 500 d old free-swimming larvae were added. The larvae soon settled on the substrate in these containers and metamorphosed to juveniles. The 6 containers were kept in the dark at 20°C. Water in the containers was gently aerated. The worms were exposed to 2 different levels of sulfide by adding 0.1 and 0.2 g of sodium sulfide crystals ($Na_2S \cdot 9H_2O$) to 30 ml sea water (low- and high-sulfide treatments, respectively), plus 1 control to which no sulfide was added. These solutions (13.9 mM in low-sulfide treatments, 27.8 mM in high-sulfide treatments) were gently injected into the sediments twice daily, beginning 3 d after the start of the experiments. In the control containers, unamended sea water was injected in the same manner. Each treatment was duplicated. The experiment lasted 6 wk. At the end of the experiment, 3 sediment cores (diam. = 36 mm) were taken from each container. One was used to determine AVS levels (Gastec, No. 201H) and stable-isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of the sedimentary organic matter. The latter were determined on a Finnigan Delta-S stable-isotope mass spectrometer coupled to a Heraeus elemental analyzer through a Finnigan cryogenic gas-trapping box. Sediments were treated with dilute HCl prior to analysis to remove carbonates and then dried (50°C). The other 2 cores were fixed in 10 % formalin solution containing Rose Bengal dye and sieved on a 125 µm mesh. The worms were counted and the maximum widths of the thoracic segments (cf. Tsutsumi & Kikuchi 1984) were measured with a stereoscopic microscope image-analysis system. This system included a CCD (charged-coupled device) camera (Sony, DXC-930) on a microscope (Olympus, SZH), a personal computer (Apple Macintosh, Quadra 700) with a frame-grabber card (Data Translation, Quick-Capture), and image-analysis software (NIH Image Version 1.55).

The remaining sediment in each container was sieved on a 125 µm sieve. The worms were removed and maintained in individual petri dishes with sea water until they had excreted all faecal pellets. The worms were then dried at 50°C, ground to a fine powder, and their stable-isotope compositions determined.

Expt 2 (Table 2). This experiment utilized additions of $Na_2{}^{13}CO_3$ to the water in the culture containers in a dark environment to demonstrate the fixation of CO_2 by chemoautotrophic bacteria within the sediment. Water (19.6 l) was sparged with N_2 gas for 24 h, removing a portion of the dissolved inorganic carbon from the water. The sparged water was then equally divided into 2 capped beakers. Then, 2.3 g of Na_2CO_3 containing 99 % ^{13}C (Isotech, Inc.) was added to 1 beaker of sparged water, while the other received an equivalent amount of Na_2CO_3 with natural isotopic abundance. Both beakers were then thoroughly shaken.

Two sets of experimental containers were prepared. One set of 4 containers (Group 1: A–D) contained sea water of natural isotopic abundance. The second set of 4 containers (Group 2: A–D) contained 99 % $Na_2{}^{13}CO_3$ sea water. Each was treated as follows: A and C containers had no

Table 1. Expt 1. Concentrations of acid-volatile sulfide (AVS) of sediments and percent survival of worms after 6 wk

	Treatment					
	Control-1	Control-2	Low sulfide-1	Low sulfide-2	High sulfide-1	High sulfide-2
AVS of sediment (mg g^{-1} dry sediment)	0.39	0.45	0.53	0.65	0.67	0.79
Survival %	40.6	57.6	70.6	74.2	62.4	65.8

Table 2. Expt 2. Experimental conditions of 4 containers in Treatment Groups 1 and 2

	Group 1				Group 2			
	A	B	C	D	A	B	C	D
Sodium carbonate added to the water	Natural	Natural	Natural	Natural	99% ^{13}C	99% ^{13}C	99% ^{13}C	99% ^{13}C
Daily dosing of sodium sulfide	None	None	0.2 g, twice	0.2 g, twice	None	None	0.2 g, twice	0.2 g, twice
Initial no. of *Capitella* sp. I larvae	None	500	None	500	None	500	None	500

Capitella sp. I; B and D containers had 500 individual *Capitella* sp. I larvae; A and B containers did not receive daily sulfide treatments; C and D containers received sulfide treatments.

The experiment lasted for 8 wk. Sulfide was administrated twice daily by the injection of 0.2 g Na_2S per 10 ml seawater solution into the sediment. Incubation temperature was 15°C. The water in the culture containers was exchanged weekly. The $\delta^{13}C$ of the sediments and *Capitella* sp. I was determined with a Europa Scientific ANCA-GSL elemental analyzer coupled with a Europa 20-20 stable-isotope ratio-mass spectrometer in continuous-flow mode. All other analytical procedures were identical to those in Expt 1.

RESULTS

Expt 1

AVS and survival

Table 1 shows the levels of AVS within the sediments and the percent survival of *Capitella* sp. I after 6 wk. AVS includes hydrogen sulfide and metal sulfides. Even in the controls, where we did not add Na_2S solution to the sediments, AVS was present at concentrations of 0.39 and 0.45 mg g^{-1} dry sediment. The AVS levels of the sediments increased proportionally with increasing concentrations of Na_2S solution injected into the sediments (0.53 and 0.65 mg g^{-1} dry sediment in the low-sulfide treatments, 0.67 and 0.79 mg g^{-1} dry sediment in the high-sulfide treatments). The elevated AVS levels in the 2 sulfide treatments caused no apparent negative effects on the survival of the worms. In fact, the survival rates were highest in the low-sulfide containers (70.6 and 74.2%) and were higher in both of the sulfide treatments than in the controls (40.6 and 57.6%). However, the difference in survival between treatments was not statistically significant (Welch's *t*-test, $t[0.975] = 12.70$, $p = 0.28$).

Growth

When *Capitella* sp. I larvae settle onto the sediment, the maximum thoracic width is approximately 0.15 mm (cf. Tsutsumi & Kikuchi 1984, Tsutsumi 1987). After 6 wk, distinct differences in worm size were observed between the controls and the sulfide treatments (Figs. 1 & 2). In the controls, the mean maximum thoracic width of the worms was less than 0.3 mm, and no worms exhibited signs of sexual maturity (development of genital hooks in males and ovaries in females). In the low- and high-sulfide treatments, the mean maximum thoracic widths were 0.53 to 0.58 mm. These differences in body size between the controls and the sulfide treatments were statistically significant (ANOVA, $p < 0.01$). This species becomes sexually mature when the maximum thoracic width is 0.4 to 0.5 mm (Tsutsumi & Kikuchi 1984, Tsutsumi 1987). In the sulfide treatments, some worms had already

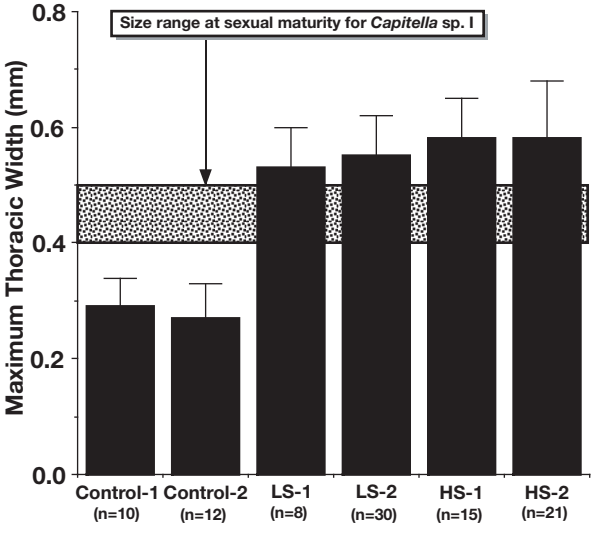

Fig. 1. *Capitella* sp. I. Body size, as maximum width of thoracic segments, after 6 wk, in duplicates of 3 treatments (n = total number of worms captured in 2 cores, surface area = 10.2 cm^2, taken from each container). LS: low sulfide; HS: high sulfide

Fig. 2. *Capitella* sp. I. Fixed specimens after 6 wk. Right: control juvenile, 0.32 mm max. thoracic width; left: high-sulfide treatment female in a brood tube, 0.58 mm max. thoracic width

reproduced. We collected brooding females with brood tubes from the high-sulfide treatments (Fig. 2).

Stable isotopes

Table 3 shows the results of the stable-isotope analysis of the sediments and the worms. Prior to the start of the experiment, the $\delta^{13}C$ and $\delta^{15}N$ values of the sediments were –21.7 and +8.7‰, respectively. After 6 wk, $\delta^{13}C$ values (–21.4 to –21.2‰) were slightly enriched in the sediments in all 3 treatments (0.3 to 0.5‰ higher than initial). Distinct carbon-isotope differences were found among worms from the 3 experimental treatments. The 2 sets of control worms were isotopically enriched relative to the sediment (by 0.9 and 1.3‰ in $\delta^{13}C$). In contrast, worms in the low-sulfide treatments were depleted (0.8 and 0.5‰) and were 1.8‰ depleted relative to the control worms. Worms in the high sulfide treatments were 3.8 and 2.2‰ depleted relative to the sediment.

The $\delta^{15}N$ values of the sediments were slightly depleted in the controls (–0.4‰) and in the low-sulfide treatments (–0.2‰) relative to initial values and were slightly enriched in the high-sulfide treatments (+0.1‰). Worms were enriched in ^{15}N relative to the sediments by 2.0 to 2.9‰ in all 3 treatments.

Table 3. Expt 1. Carbon and nitrogen isotope ratios ($\delta^{13}C$, $\delta^{15}N$) of sediments and *Capitella* sp. I. Asterisk indicates single measurement, others are mean of 2 measurements

	Treatment					
	Control-1	Control-2	Low sulfide-1	Low sulfide-2	High sulfide-1	High sulfide-2
$\delta^{13}C$ of bulk sediment						
Initial	–21.7	–21.7	–21.7	–21.7	–21.7	–21.7
6 wk later	–21.3	–21.2	–21.2	–21.2	–21.3	–21.4
$\delta^{13}C$ of worms 6 wk later	–20.2*	–19.9	–22.0*	–21.7*	–25.1	–23.6*
Worms-sediment	+0.9	+1.3	–0.8	–0.5	–3.8	–2.2
$\delta^{15}N$ of bulk sediment						
Initial	+8.7	+8.7	+8.7	+8.7	+8.7	+8.7
6 wk later	+8.1	+8.5	+8.6	+8.3	+7.9	+9.7
$\delta^{15}N$ of worms 6 wk later	+10.7	+10.1	+12.3	+12.4	+10.4	+11.2
Worms-sediment	+2.6	+1.6	+3.7	+4.1	+2.5	+1.5

Expt 2

Worm growth rates and AVS levels were similar to those found in Expt 1, with a significant enhancement of growth in the treatments that received sulfide additions (see Treatments 1-D and 2-D in Table 4). In treatments to which unlabeled sodium carbonate (Group 1) had been added to the sea water, the carbon and nitrogen isotopic signatures of the sediments were relatively invariant throughout the experiments. However, the worms in Treatment 1-D (with sulfide) that grew to adulthood were depleted in ^{13}C relative to the sediment 8 wk later (–2.3‰ in $\delta^{13}C$), a similar depletion to that seen in Expt 1 (Table 3).

In Group 2, which received ^{13}C amendments, the sediments were enriched in ^{13}C, presumably due to bacterial chemosynthesis. The fixation of $^{13}CO_2$ within the sediments was markedly enhanced by the presence of Na_2S (Treatments 2-C, 2-D) and was further enhanced by the presence of *Capitella* sp. I (Treatment 2-D). The carbon-isotope signature of the worms in Treatment 2-D reached +5218.2‰ after 8 wk. This extreme ^{13}C enrichment in the worms compared with the sediment (+258.7‰ in $\delta^{13}C$ after 8 wk) suggests that the enhanced growth of the worms in the sulfide treatments relied on the selective ingestion of chemosynthetically produced organic matter from the sediments.

DISCUSSION

In Expt 1, control *Capitella* sp. I showed a relatively high mortality

Table 4. Expt 2. Acid-volatile sulfide (AVS) of sediments, growth of *Capitella* sp. I, and carbon and nitrogen isotope ratios ($\delta^{13}C$, $\delta^{15}N$) of sediments and worms. All results are means of 2 measurements

	Treatment							
	1-A	1-B	1-C	1-D	2-A	2-B	2-C	2-D
AVS after 8 wk	0.01	0.01	0.27	0.33	0.02	0.00	0.21	0.19
Mean body size (mm) (max. width of thoracic segments)	–	0.16	–	0.41	–	0.14	–	0.55
$\delta^{13}C$ of sediment								
2 wk later	–20.5	–20.7	–20.4	–20.3	+1.9	+5.3	+12.5	+28.9
4 wk later	–20.6	–20.8	–20.7	–20.6	–0.2	–2.5	+8.5	+75.9
6 wk later	–20.9	–20.7	–20.6	–20.9	+6.2	–4.9	+17.0	+206.4
8 wk later	–20.7	–20.7	–20.9	–20.7	+5.5	–5.4	+40.9	+258.7
$\delta^{13}C$ of worms 8 wk later	–	–[a]	–	–23.0	–	–[a]	–	+5218.2
$\delta^{15}N$ of sediment								
2 wk later	+8.3	+8.1	+8.3	+7.8	+8.1	+7.8	+7.8	+8.6
4 wk later	+7.9	+7.8	+7.9	+7.4	+8.1	+7.6	+7.8	+7.7
6 wk later	+7.9	+8.1	+7.9	+8.0	+8.4	+8.2	+7.9	+8.3
8 wk later	+8.0	+7.4	+7.7	+7.7	+7.8	+7.8	+8.5	+7.6
$\delta^{15}N$ of worms 8 wk later	–	–[a]	–	+6.6	–	–[a]	–	+8.2

[a]Collected biomass of worms was too small for determination of carbon and nitrogen isotope ratios

(mean = over 50%; Table 1) and depressed growth (Fig. 1), probably due to a shortage of food. In the sulfide treatments, worms were cultured under the same conditions as the controls except for the daily supply of Na_2S solution to the sediment. Although even micromolar to millimolar levels of hydrogen sulfide are toxic to many aquatic organisms (Oseid & Smith 1974a,b, Thompson et al. 1991, Vismann 1991, 1996, Bagarinao 1992), survival rates of the worms were higher (Table 1) and growth was promoted (Fig. 1). The majority of worms in the sulfide treatments reached maturity within 6 wk, and some of them reproduced, as we have observed in field populations in organically enriched areas and in laboratory colonies with ample food (Tsutsumi 1987, 1990, Tsutsumi et al. 1990). Unfortunately, we were not able to measure pH, DO (dissolved oxygen) and hydrogen sulfide levels in the interstitial water of the sediment, and therefore we do not know how much of the AVS that we measured was in toxic form (Vissman 1996) or the exact location within the sediments of conditions suitable for chemoautotrophy. However, the higher survival rates and enhanced growth of the worms in the sulfide treatments versus controls indicate that the addition of Na_2S solution to the sediment did not have negative effects on the worms, but rather played a role in supplying additional food for them.

Because the worms were maintained in the dark, in sediment of low organic content with no addition of organic matter, we believe that the only source of organic matter available for the enhanced growth of the worms in the sulfide treatments was the organic matter produced by chemoautotrophic bacteria utilizing hydrogen sulfide. Isotopic fractionation by chemoautotrophic bacteria during carbon fixation (Degens 1969, Ruby et al. 1987, Conway et al. 1989, Conway & McDowell-Capuzzo 1992) may be at least as large as that exhibited by phytoplankton (Goericke 1994, Fry 1996). We did not measure the $\delta^{13}C$ of dissolved inorganic carbon of the sea water used for our experiments, but assume it to have been typical of average seawater, i.e., 1 ± 1‰. We reasoned that organic matter produced by chemoautotrophs may have a lighter isotopic signature than that of bulk sedimentary organic matter of phytoplankton origin (Degens 1969, Ruby et al. 1987). If so, then worms that rely on chemosynthetic production should also be distinctly labeled, since the $\delta^{13}C$ values of animals and their diet tend to differ by only about 1‰ (Michener & Schell 1994).

In Expt 1 (Table 3), we did not determine the carbon-isotope ratios of the juveniles just after settlement at the start of the experiments. However, the initial biomass derived from these juveniles should not have influenced the carbon-isotope ratios of the worms after 6 wk. The mean body dry weight of the juveniles at settlement, based on their maximum thoracic width of 0.15 mm (Table 4) was estimated to be 2.3 µg (cf. Tsutsumi & Kikuchi 1984, Tsutsumi 1987). After 6 wk, the mean body weight of the worms was similarly estimated to be 10.1 µg in the controls, 68.4 µg in the low-sulfide treatments, and 87.1 µg in the high-sulfide treatments. Even the mean dry weight of the control worms after 6 wk was 4.4 times heavier than the juveniles at settlement.

The carbon-isotope ratios of the control worms was slightly higher (approximately 1‰ for $\delta^{13}C$ values) than the bulk sediment in which they fed. This increase in the $\delta^{13}C$ ratio is within the range of trophic enrichment between animals and their diet (Michener & Schell 1994). In contrast, worms in the sulfide treatments had lower $\delta^{13}C$ values than the bulk sediment,

with the values in the high-sulfide treatments being lower than those in the low-sulfide treatments. The distinctive $\delta^{13}C$ signature of worms from the sulfide treatments indicates that the worms in the sulfide treatments did not utilize the same carbon source of organic matter as the control worms, even though we did not add organic matter to any of the treatments. We deduce that promotion of growth of the worms in the sulfide treatments resulted from the exploitation of organic matter that was chemosynthetically produced.

Chemosynthetic production of organic matter was, however, not detectable from $\delta^{13}C$ values of the bulk sediments. In fact, sediments from all treatments were slightly enriched in ^{13}C, possibly due to mineralization of ^{12}C-rich organic matter. Given the relatively large pool of organic matter initially present in the sediment (approximately 9 g of organic carbon in the mud per container), it is unlikely that chemosynthetically produced organic matter would be detectable, unless it were either very abundant or very different, isotopically, from bulk organic matter.

In Expt 2, the results for treatments that did not receive ^{13}C amendments (Group 1; Table 4), were similar to those of Expt 1, i.e., the worms showed enhanced growth with Na_2S amendments and were depleted in $\delta^{13}C$ relative to the sediments (Treatment 1-D). These results agree with the results of the sulfide-treated sediments and worms in Expt 1. In Treatment 2-C, in which ^{13}C-labeled dissolved inorganic carbon was added to the sea water and sediments received Na_2S amendments, the stable carbon-isotope signature of sedimentary organic matter (+40.9‰ after 8 wk) indicated enhanced CO_2 fixation in the sediments. Thus, the chemosynthetic production that occurred in the sediments with Na_2S amendments was detectable through the isotopic enrichment of carbonate in the sea water.

By enriching the dissolved inorganic carbon with ^{13}C in Expt 2, we were able to trace the trophic processes from dissolved inorganic carbon to chemoautotrophic bacteria to *Capitella* sp. I. Higher $\delta^{13}C$ measurements of the sediment to which Na_2S solution was added indicate the presence of chemoautotrophic bacteria (Treatments 2-C and 2-D), and the extremely high ^{13}C measurements of *Capitella* sp. I (+5218.2‰ in $\delta^{13}C$, 8 wk later: Treatment 2-D) indicate selective utilization of that bacterial food source.

The further enrichment of ^{13}C values of sedimentary organic matter in the presence of *Capitella* sp. I (compare Treatments 2-C and 2-D) suggests that the burrowing or irrigation activity of the worm may stimulate sulfur-oxidizing bacteria by increasing the surface area of the O_2/S_2 interface (particle reworking and micro-environmental heterogeneity; Aller 1982). In a follow-up study (unpubl. data), we first cultured *Capitella* sp. I on agar plates, using a vital staining technique of bacteria with INT, 2(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (cf. Zimmermann et al. 1978). We confirmed that bacterial activities were apparently increased around the burrow surface of the worms compared with surrounding sediments. There may be a mutualism, whereby *Capitella* sp. I stimulates the production of organic matter by chemoautotrophs.

We do not know the extent to which *Capitella* species utilize chemosynthetically produced organic matter in organically enriched sediments in the field. However, the results of our study demonstrate that *Capitella* sp. I shows enhanced survival and growth in enriched sulfide conditions, facilitated by its utilization of a novel source of organic matter, and in an apparently symbiotic relationship with chemoautotrophic bacteria. Although *Capitella* species commonly occur at extremely high densities in organically enriched sediments in coastal areas throughout the world (Pearson & Rosenberg 1976, 1978), the reasons for their association with organically enriched sediments have remained unclear. The results of our study suggest that an additional supply of labile organic matter from chemosynthetic production may be an important factor controlling the distribution and the population dynamics of *Capitella* species.

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