Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web

Cindy Lee Van Dover*, J. F. Grassle†, Brian Fry‡, Robert H. Garritt‡ & Victoria R. Starczak§

* Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA
† Institute for Marine and Coastal Sciences, Cook College, Rutgers University, New Brunswick, New Jersey 08903, USA
‡ Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA
§ Applied Ocean Physics and Engineering Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

CHRONIC pollution of the open ocean has occurred since 1986 through disposal of municipal sewage sludge at a deep-water (~2,500 m) dumpsite off the coast of New Jersey. Dispersal and dilution of sewage particulates in surface waters were presumed to be sufficient to prevent or minimize accumulation of detectable amounts of sewage-derived material on the sea floor. Using stable isotope ratios of carbon, nitrogen and sulphur as tracers of sewage-derived organic material, we show here that this material reaches the sea floor and enters the benthic food web, specifically through surface-deposit feeding activities of the urchin, *Echinus affinus* and the sea cucumber, *Benthodytes sanguinolenta*.

Megascale pollution of the open ocean 185 km off the coast of New Jersey was maintained from March 1986 to July 1992 through dumping of municipal sewage sludge at a rate of 8–9 million wet metric tons per annum[1]. The potential for perturbation and/or environmental degradation of benthopelagic and benthic ecosystems within and downstream of the dumpsite, designated Deep-Water Dumpsite 106 (DWD-106), was originally de-emphasized by planners, whose initial monitoring strategies focused exclusively on the water column[2,3]. But subsequent characterization of sludge particle sizes and settling dynamics[4] suggests that initial interpretations of a benthically innocuous fate for sewage sludge may be erroneous. New models using historic current meter and particle settling-velocity data[5] predict measurable sea-bed loading of sewage-derived particulates up to 350 km downstream of the dumpsite. Recent studies of sediments in the region indicate that levels of sewage indicators are increased in the dumpsite area[6–9].

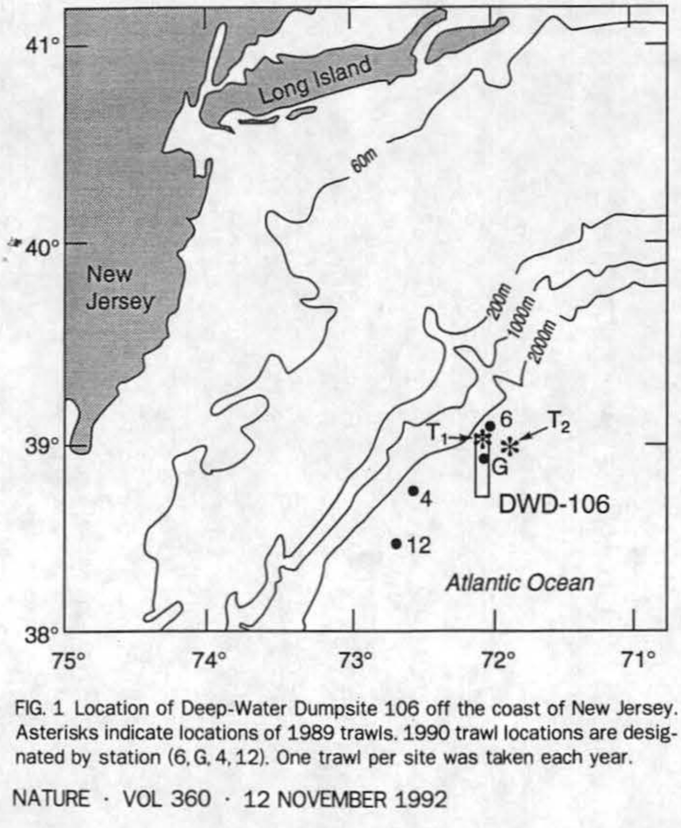

FIG. 1 Location of Deep-Water Dumpsite 106 off the coast of New Jersey. Asterisks indicate locations of 1989 trawls. 1990 trawl locations are designated by station (6, G, 4, 12). One trawl per site was taken each year.

TABLE 1 Isotope composition of sewage sludge and of open-ocean, phytoplankton-derived particulate organic material

$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$	Location	Reference
Sewage-derived organic material				
−24.7	−1.1	+2.3	Middlesex, NJ	this study
−23.2	+6.1	+2.3	Bergen, NJ	this study
−21.4	+7.2	+3.5	Yonkers, NY	this study
−23.0	+3.2	+4.0	NY/NJ	24
−26.0			NY Bight	12
	+2.5	0.0	Whites Point, CA	25
−16.5	+1.8		Whites Point, CA	15
−23.5			Whites Point, CA	26
−23.7		+3.0	Providence, RI	this study
Phytoplankton-derived particulate organic material				
−21.7	+6.1		N. Atlantic 38° 57.79′ N 71° 56.7′ E sediment top 1 cm 2,419 m	this study
−21.6			Sedimentary POC Western N. Atlantic 3,471 m	27
	+5 to +15		Deepwater Pacific PON	28
	+5 to +7		Sargasso Sea Nisken and Pump Sample PON 1,000 m	29
		+17 to +21	Marine algae POS	16, 23, 30

The magnitude of organic loading 50 km downstream of the dumpsite is estimated to be of the same order as seasonal pulses of phytodetritus reaching the abyssal sea floor after surface blooms (F. Sayles, personal communication, based on estimates of sewage flux to the sea floor[3,5] and background flux of organic carbon[10]). Such a doubling of food resources at DWD-106 is likely to have a detectable effect on the deep-sea biotic community. Evidence for entry of sewage-derived organic material into the deep-sea food web would provide a conclusive test of this hypothesis.

We chose to exploit the natural stable-isotope compositions of organic carbon, nitrogen and sulphur in sewage sludge as tracers for sewage-derived organic matter (SDOM)[11–15]. If sewage sludge does not enter the abyssal food web, megafaunal invertebrate and fish species trawled from areas within and immediately downstream of the disposal site should have similar carbon, nitrogen and sulphur isotope compositions as organisms from reference areas upstream of the site along similar depth contours and sediment types. This null hypothesis assumes that the only difference between dumpsite and reference areas is the potential for sewage-derived organic enrichment within and downstream of the dumpsite, an assumption consistent with submersible observations and our present understanding of the seafloor environment within the region. To establish end-member values (Table 1), we analysed carbon and nitrogen isotope compositions of plankton-derived organic material (PDOM) in the surface layer of sediment from a site upstream

TABLE 2 Carbon, nitrogen and sulphur isotope compositions (‰) of megafaunal species collected from dumpsite and reference trawls

Species		Tissue	Site	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
Flabellum angulare	Solitary coral	Soft parts	DWD-106	−19.9±0.3 (5)	+10.3±0.5 (5)	+17.6±0.3 (3)
			Reference	−19.7±0.4 (8)	+10.5±0.3 (8)	+18.1±1.6 (3)
				[0.42]	[0.33]	[0.62]
Echinus affinus	Sea urchin	Gonad	DWD-106	−22.1±1.1 (10)	+5.5±1.2 (10)	+15.3±1.2 (9)
			Reference	−21.0±0.6 (3)	+9.9±0.3 (3)	+17.4±0.6 (3)
				[0.13]	[<0.000]***	[0.01]*
Brissopsis mediterranea	Heart urchin	Gonad	DWD-106	−21.7 (1)	+11.0 (1)	—
			Reference	−21.1 (1)	+10.9 (1)	—
Pectinaster filholi	Seastar	Gonad	DWD-106	−20.2±0.6 (3)	+11.8±2.0 (3)	+16.9±0.3 (3)†
			Reference	−21.0±0.5 (3)	+12.6±3.1 (3)	+15.7±1.6 (3)†
				[0.11]	[0.74]	[0.34]
Benthodytes sanguinolenta	Sea cucumber	Body wall	DW-106	−17.1±0.8 (3)	+7.7±0.8 (3)	+17.1±0.4 (3)
			Reference	−14.7±0.7 (3)	+11.2±0.5 (3)	+18.7±0.2 (3)
				[0.02]*	[0.003]**	[0.002]**
Hedingia albicans	Sea cucumber	Body wall	DWD-106	—	—	+17.6±0.3 (3)
			Reference	—	—	+17.9±0.3 (3)
						[0.38]
Glyphocrangon sculpta	Shrimp	Muscle	DWD-106	−16.0 (1)	+13.0 (1)	—
			Reference	−15.9 (1)	+13.2 (1)	—
Munidopsis sp	Squat lobster	Muscle	DWD-106	−17.7 (1)	+10.5 (1)	
			Reference	−17.6 (1)	+10.8 (1)	—
Antimora rostrata	Deep-sea cod	Muscle	DWD-106	−18.0 (1)	+12.4 (1)	—
			Reference	−18.5±0.8 (3)	+12.1±0.3 (3)	—
Lionurus carpinus	Rat tail	Muscle	DWD-106	−18.0±0.6 (2)	+12.8±0.7 (2)	—
			Reference	−17.5 (1)	+12.7 (1)	

Values are given as mean ±s.d. (n); analytical error is ±0.1‰ except for small sulphur samples indicated by †, where the analytical error is ±0.25‰. When $n \geq 3$, isotope compositions were compared between dump site and reference site using a two sample t-test. Significant results are indicated with asterisks; P values are given in brackets. Statistical analysis assumed that individuals were sampled randomly from each site.

of the dumpsite; sulphur and additional carbon and nitrogen isotope data for PDOM are reported from the literature. Isotope composition of sewage sludge was analysed in February 1991 samples from contributing metropolitan New York and New Jersey treatment facilities (Table 1). Comparison of PDOM and SDOM indicates that sulphur should provide the strongest signal ($|\Delta\delta_{PDOM\text{-}SDOM}| = 11.5$ to 18.7‰), carbon the weakest signal ($|\Delta\delta_{PDOM\text{-}SDOM}| = 0.3$ to 3.1‰), with nitrogen intermediate ($|\Delta\delta_{PDOM\text{-}SDOM}| = 1.1$ to 7.2‰). The large difference in the sulphur isotope composition of PDOM and SDOM is attributable to the +2 to +6‰ δ^{34}S composition of continental vegetation and the +17 to +21‰ values of marine plankton[16].

We initially screened ten megafaunal species common to trawls from DWD-106 and a reference site collected in September 1989 (Fig. 1) for carbon, nitrogen, and/or sulphur isotope composition (Table 2). For five species, isotope analyses were done on tissues from 3 or more individuals per site. Carbon isotope compositions of dumpsite and reference pairs for four of these species were not significantly different. There was a significantly more negative δ^{13}C composition in the sea cucumber, *B. sanguinolenta*. The ~2.5‰ depletion of ^{13}C in the dumpsite *B. sanguinolenta* is consistent with a contribution of SDOM to its diet. Between-species differences in δ^{13}C values may be attributed to the biochemical composition of the tissues analysed: lipid-rich tissues such as gonad and the soft parts of the solitary coral (predominantly gonad) are isotopically lighter ($\delta^{13}C = -19.7$ to -21.9‰) than protein-rich tissues of body wall and muscle ($\delta^{13}C = -14.7$ to -18.5‰) owing to discrimination towards ^{12}C during lipogenesis[17,18].

Eight of ten species analysed for nitrogen and/or sulphur isotope composition show no difference between dumpsite and reference individuals within species (Table 2). In two species, *E. affinus* and *B. sanguinolenta*, there are significant differences in δ^{15}N and δ^{34}S values between dumpsite and reference individuals. These differences are consistent with contributions of ^{15}N- and ^{34}S-depleted SDOM in the diet of dumpsite individuals. Both *E. affinus* and *B. sanguinolenta* are surface-deposit feeders. In contrast, the other megafaunal species surveyed are variously suspension feeders (*Flabellum angulare*), subsurface deposit feeders (*Brissopsis mediterranea*, *Hedingia albicans*), or motile predators or scavengers (*Glyphocrangon sculpta*, *Munidopsis* sp., *Antimora rostrata*, *Lionurus carpinus*, and possibly *Pectinaster filholi*). Suspension feeders feed in the water column where SDOM does not accumulate. In subsurface sediments, concentrations of SDOM are reduced to undetectable levels, based on measurement of sewage indicators[6,7]. Predatory or scavenging shrimp, crabs and fish feed on a variety of benthic invertebrates, some of which may have access to SDOM. The foraging ambit of these motile predators, however, may take them beyond the area influenced by sludge disposal, thereby reducing their opportunities for uptake of SDOM. Surface deposit-feeders, *E. affinus* in particular, are known to be attracted to aggregations of food[19], including seasonal pulses of organic matter[20,21] and, as shown here, SDOM should be most readily available to surface-deposit-feeding species.

Individuals of *E. affinus* from the reference area, where only organic material from natural sources is available, show less variability in isotope composition than dumpsite urchins (Table 2). Isotope variability within the dumpsite population may reflect microgeographic differences in deposition and availability of SDOM versus PDOM[6-9]. There may also be an age-dependent variation if turnover rate of tissues within an individual is longer than the period of dumping; tissues of individuals populating the dumpsite area before the start of dumping may reflect a mixture of pre- and post-dumping trophic conditions. We examined this latter possibility by analysing δ^{34}S of urchin size series trawled in August 1990 from dumpsite and reference areas. We expect and find no linear relationship between size and δ^{34}S composition in reference urchins and a significant positive linear relationship between size and δ^{34}S composition of dumpsite urchins, reflecting an increased contribution of SDOM to smaller, more rapidly growing individuals (Fig. 2*a*).

Using a simple, two-source mixing model, we can estimate the relative importance of sewage-derived sulphur compounds in the metabolism of dumpsite urchins: $\delta^{34}S_{\text{dumpsite urchin}} = f\delta^{34}_{\text{sewage sludge}} + (1-f)\delta^{34}S_{\text{phytodetritus}}$, where f is the proportion of sewage-derived organic sulphur in the diet of the urchin and δ^{34}S values for urchins, sludge and phytodetritus are 12.2, 3.0 and 17.4%, respectively. Because we see little difference between

$\delta^{34}S$ of phytoplankton and of reference urchins consuming PDOM, we infer that there is little fractionation of sulphur isotopes in organic material as it sinks through the water column. Other laboratory and field studies indicate that little sulphur isotope fractionation occurs during decomposition of organic material or in off-shore ecosystems[22,23]. Further, $\delta^{34}S$ of sediment recovered from traps moored 250 m above the bottom within 20 nmi of the dumpsite in 1991 approached $\delta^{34}S$ values of the sludge dumped at the site[24]. We thus use the mean $\delta^{34}S$ for sewage sludge from New York and New Jersey treatment plants (Table 1) as one end-member source in the mixing model. The second end-member, $\delta^{34}S_{phytodetritus}$, is approximated by the average $\delta^{34}S$ of reference urchins in 1989 (Table 2). Using this model, we find that as much as 35% of the sulphur in gonadal tissues of dumpsite urchins may be derived from SDOM. This is likely to be a conservative value, inasmuch as the most recent model of sludge particle flux[5] shows a maximum flux in a region 10 km west of the dumpsite, a location from which we have not yet collected urchins.

In August 1990, additional *E. affinus* were collected by trawls from a reference site (station 6) north and upstream of the dumpsite, from a location in the dumpsite (station G), and from two stations (stations 4, 12) located about 70 km to the southwest, or downstream of the dumpsite (Fig. 1). From each of these stations, we selected urchins of roughly equal size and analysed the $\delta^{15}N$ and $\delta^{34}S$ compositions of their gonads. We again found clear, two-dimensional separation of dumpsite and reference urchins (Fig. 2*b*). Within-site variability was considerably reduced in the 1990 samples, which most likely reflects the selection of more uniform-sized urchins for analysis.

Based on these isotope data, we conclude that SDOM reaches the deep-sea floor and enters the benthic food web as a result of consumption by surface-deposit feeders, specifically the sea cucumber *B. sanguinolenta* and the urchin *E. affinus*. Our use of stable isotope tracers was limited to large, readily collected megafaunal species with sufficient organic tissue to allow analysis on an individual basis. We anticipate that other surface-deposit-feeding organisms, including infaunal macroinvertebrates (such as polychaetes, molluscs, crustaceans) will prove to have access to SDOM.

Contrary to earlier assumptions, chronic disposal of sewage sludge in the open ocean does impact the benthic ecosystem underlying and downstream of the disposal site. It is likely that long-term disposal programs in the open ocean will result in a restructuring of the benthic community in favour of species such as *E. affinus* and *B. sanguinolenta*, which can readily exploit particulate organic enrichments. With the cessation of dumping in July 1992, the data reported here serve as a critical benchmark for assessment of recovery of the benthic food web to pre-dumping conditions. □

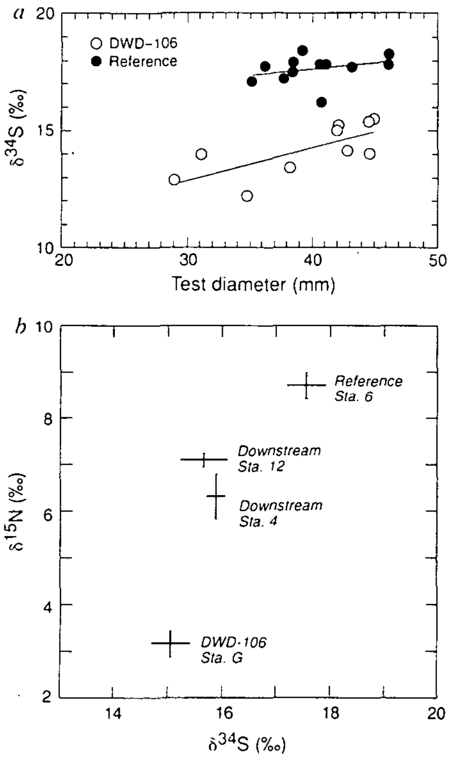

FIG. 2 *a*, $\delta^{34}S$ (‰) of urchin (*Echinus affinus*) gonads versus urchin test diameter (mm) for 1990 trawl samples. Reference urchins had test diameters ranging from 35 to 46 mm, corresponding to an age range of ~15 to 25 years, based on a growth model generated for a population of *E. affinus* in the Rockall Trough (northeastern North Atlantic) at 2,200 m[31]. Under the same growth model, dumpsite urchins ranging in diameter from 29 to 45 mm correspond to an age range of 10 to 25 years. Thus all of the urchins analysed are likely to have experienced pre-dumping trophic conditions, with smallest animals experiencing a greater proportion of growth since the initiation of dumping in 1986. Isotope compositions are expressed using the delta notation[16] and were determined using standard techniques[32,33]. Reference regression equation and statistics: $y=15.647+0.049x$; $F_{1,10}=0.97$; $R^2=0.088$; $P=0.3488$. Dumpsite regression equation and statistics; $y=8.807+0.136x$; $F_{1,8}=8.47$; $R^2=0.514$; $P=0.02$. A greater number of smaller individuals were analysed from the dumpsite than from the reference site. A regression analysis was performed using only individuals with test diameters >34 mm. The resulting regression ($y=2.973+0.272x$; $R^2=0.712$; $F_{1,6}=14.81$; $P=0.008$) was significant, indicating that the results in the original analysis are robust to the deletion of individuals 34 mm or smaller. *b*, $\delta^{34}S$ *vs* $\delta^{15}N$ (‰; ±s.e.) for urchin (*E. affinus*) gonads collected in 1990 trawls; $n=5$ for all data sets except Station 12, where $n=4$ for sulphur isotope analysis. Station locations are shown in Fig. 1. Mean test diameters (±s.d.): Station G: 43.8±1.3 mm; Station 6: 43.3±2.7 mm; Station 4: 36.4±2.7 mm; Station 12: 44.0±2.5 mm. Tukey's Multiple Comparisons test on the mean $\delta^{34}S$ and $\delta^{15}N$ values from the four stations sampled shows that $\delta^{34}S$ and $\delta^{15}N$ values of dumpsite and reference urchins are significantly different and that urchins from downstream stations 4 and 12 had nitrogen isotope compositions intermediate to those of dumpsite and reference urchins. These results are consistent with modelling predictions of significant but decreasing flux of SDOM to the seafloor at distances to the southwest of 50 km and more[5]. There was no difference in mean $\delta^{34}S$ among urchins sampled from the dumpsite and from intermediate stations 4 and 12. Mean $\delta^{15}N$ values of dumpsite urchins decreased significantly from 5.5‰ in 1990 (2-sample *t*-test; $t=5.04$; $P=0.0002$; d.f.$=13$) but no significant difference in $\delta^{34}S$ was detected in the one-year interval ($t=0.33$; $P=0.750$; d.f.$=11$).

Received 11 August; accepted 22 September 1992.

1. Robertson, A. & Redford, D. *Eos Trans. Am. Geophys. Union* **73**, 164 (1992).
2. O'Connor, T. P., Walker, H. A., Paul, J. F. & Bierman, V. J. *Mar. envir. Res.* **16**, 127-150 (1985).
3. O'Connor, T. P., Okubo, A., Champ, A. & Park, K. P. *Can. J. Fish. Aqua. Sci.* **40** (suppl. 2), 228-241 (1985).
4. Lavelle, J. W., Ozturgut, E., Baker, E. T., Tennant, D. A. & Walker, S. L. *Envir. Sci. Technol.* **22**, 1202-1207 (1988).
5. Fry, V. A. & Butman, B. *Envir. Res.* **31**, 145-160 (1991).
6. Bothner, M. H., d'Angelo, W. M. & Grassle, J. F. *Eos Trans. Am Geophys. Union* **72**, 83 (1992).
7. Takada, H., Tripp, B. W., Clifford, C. H., Farrington, J. & Bothner, M. *Eos Trans. Am. Geophys. Union* **72**, 83 (1992).
8. Hill, R. T., Knight, I., Anikis, M., Straube, W. L. & Colwell, R. R. *Eos Trans. Am. Geophys. Union* **72**, 83 (1992).
9. Grassle, J. F. *Eos Trans. Am. Geophys. Union* **72**, 84 (1992).
10. Biscaye, P. E., Anderson, R. F. & Deck, B. L. *Continental Shelf Res.* **8**, 855-904 (1988).
11. Sweeney, R. E. & Kaplan, R. R. *Mar. envir. Res.* **3**, 215-224 (1980).
12. Burnett, W. C. & Schaeffer, O. A. *Est. Coastal mar. Sci.* **11**, 605-611 (1980).
13. Gearing, P. J., Gearing, J. N., Maughan, J. T. & Oviatt, G. A. *Envir. Sci. Technol.* **25**, 295-301 (1991).
14. Rau, G. H., Sweeney, R. E. & Kaplan, I. R. *Est. Coastal Shelf Sci.* **13**, 701-707 (1981).
15. Spies, R. B., Kruger, H., Ireland, R. & Rice, D. W. Jr *Mar. Ecol. Prog. Ser.* **54**, 157-170 (1989).
16. Peterson, B. J. & Fry, B. *A. Rev. Ecol. Systematics* **18**, 293-320 (1987).
17. Parker, P. L. *Geochim. cosmochim. Acta* **28**, 1155-1164 (1964).
18. van der Merwe, N. J. *Am. Sci.* **70**, 596-606 (1982).
19. Grassle, J. F., Sanders, H. L., Hessler, R. R., Rowe, G. T. & McLellan, T. *Deep Sea Res.* **22**, 457-481 (1975).
20. Tyler, P. A. *Oceanogr. Mar. Biol. A. Rev.* **26**, 227-258 (1988).
21. Tyler, P. A. & Gage, J. D. *Deep Sea Res.* **31**, 387-402 (1984).
22. Mekhtiyeva, V. L., Pankina, R. G. & Gavrilov, Y. Y. *Geochim. Int.* **13**, 82-87 (1976).
23. Fry, B. *Limnol. Oceanogr.* **33**, 1182-1190 (1988).
24. Hunt, C. D., Ginsburg, L., West, D. & Redford, D. *Eos Trans. Am. Geophys. Union* **73**, 165 (1992).
25. Sweeney, R. E., Kalil, E. K. & Kaplan, I. R. *Mar. Envir. Res.* **3**, 225-243 (1980).
26. Myers, E. P. thesis, Calif. Inst. Technol. (1974).
27. Sayles, F. L. & Curry, W. B. *Geochim. cosmochim. Acta* **52**, 2963-2978 (1988).
28. Saino, T. & Hattori, A. *Deep Sea Res.* **34**, 807-827 (1987).
29. Altabet, M. A. *Deep Sea Res.* **35**, 535-554 (1988).

30. Kaplan, I. R., Emery, K. O. & Rittenberg, S. C. *Geochim. cosmochim. Acta* **27**, 297-331 (1963).
31. Gage, J. D. & Tyler, P. A. *Mar. Biol.* **90**, 41-53 (1985).
32. Fry, B. *et al. Deep Sea Res.* **38**, 51003-51019 (1991).
33. White, J. R., Gubale, C. P., Fry, B., Owen, J. & Mitchell, M. J. *Geochim. cosmochim. Acta* **53**, 2547-2559 (1989).

ACKNOWLEDGEMENTS. We thank R. Petrecca, the National Marine Fisheries Service, and the scientists and crews of *RVs Atlantis II* and *Delaware* for help in collecting specimens; D. Pabst and colleagues for helping us obtain samples of sewage sludge; M. Bothner for sediment samples; D. Pawson and B. Hecker for assistance in identification of organisms; and K. Tholke for isotope analysis. Parts of this work were sponsored by the NOAA Sea Grant College Program Office, Department of Commerce, and WHOI Sea Grant; additional support was provided by NOAA-NURP (J.F.G.).