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Fatty Acids as Sensitive Tracers of Sewage Sludge Carbon in a Deep-Sea Ecosystem

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Dumping into the open ocean is considered a viable political option to cope with the rapidly increasing global production of municipal sewage sludge. However, before such an option can be justified, the impact of mega-scale dumping upon the marine environment must be fully addressed scientifically. Here we present a methodology whereby the impact of sewage dumping upon deep-sea invertebrates can be tracked. We examined the relative abundances and carbon isotopic compositions of membrane fatty acids of the sea urchin *Echinus affinus* from one deep-sea site heavily affected by municipal sewage sludge dumping (Sludge Max; Dumpsite DWD 106) and one marginally affected (station 13; 18 km SSE of DWD 106). Such analyses reveal marked differences linked to the relative importance of sewage sludge in diet. 18:2(*n*-6) is much more abundant in Sludge Max urchins with similar, highly ^{13}C-depleted isotopic compositions in all samples ($\delta^{13}C = -25.8$ to -27.8 ‰). Conversely, 20:5(*n*-3), is most abundant in station 13 individuals and has less negative $\delta^{13}C$ values at both sites ($\delta^{13}C = -20.7$ to -22.6 ‰). We propose that the relative abundance and stable isotopic composition of 18:2(*n*-6) and 20:5(*n*-3) can be used to indicate the importance of sewage sludge in the diet of *E. affinus*.

Introduction

The dumping of municipal sewage sludge into the marine environment is a controversial issue and has recently been one of the subjects of a major United Nations sponsored intergovernmental conference (*1*) and of a UN Global Programme of Action (*2*). One reason for the contentious nature of this issue is the limited extent of research into the impact of sewage upon deep-sea ecosystems. These are especially difficult to study due to their inaccessibility, exacerbating the lack of understanding of the effects of large-scale sewage input to the marine environment generally.

In order to address some of the fundamental questions relating to the dumping of municipal sewage sludge into the opean ocean and subsequent pollution of the deep sea, much recent research has been targeted at 'Deep Water Dumpsite (DWD) 106', 185 km SE of the coast of New Jersey (Figure 1). Municipal sewage sludge, mainly from the New York metropolis, was discharged by barges to the open ocean at DWD 106 from March 1986 to July 1992 at a rate of 8−9 million t wet weight per year. Originally the impact of this sewage was expected to be minimal with regard to pelagic and benthic ecosystems associated with the sea floor (*3, 4*). However, it has been demonstrated that sewage pollution indicators such as silver, linear alkyl benzenes, and *Clostridium perfringens* spores are significantly increased in sediments below the sites of dumping (*5*−*9*). Furthermore, evidence using bulk stable isotopes of nitrogen and sulfur has indicated that benthos from sites affected by sludge dumping incorporate sewage-derived organic matter (*10*). However, bulk carbon isotope analyses are not sufficiently sensitive enough to resolve specific dietary sources of organic matter with small isotopic differences (e.g., 2−3‰), and additional techniques are required so that the fate of sewage-derived organic carbon within deep-sea ecosystems can be tracked fully.

The structure (e.g., chain length and position of unsaturation) and relative abundance of individual lipids, such as fatty acids, allow trophic relationships of a variety of marine invertebrates to be inferred (*11*−*14*). Measurement of the stable carbon isotopic compositions of these compounds further assists in inferring dietary sources within marine ecosystems (*14, 15*). In the case of the dumpsite under study, two main primary dietary sources of carbon are potentially available to benthic invertebrates: sewage sludge and marine phytodetritus, both having distinct bulk stable carbon isotopic compositions ($\delta^{13}C$ values of −24 and −21‰, respectively; *10*). Since sediment-ingesting benthic invertebrates, such as the sea urchin *Echinus affinus*, play a key role in the cycling of organic detritus on the sea floor, incorporation of sewage sludge-derived carbon by such organisms in heavily polluted sites is likely to be distinguished by lipid $\delta^{13}C$ values more negative than $\delta^{13}C$ values of corresponding lipids from a non-polluted site (where carbon ultimately of a phytoplankton source is relatively more abundant).

The aim of this study has been to explore the applicability of a combined approach, using relative abundances and stable isotopic compositions of individual gonad membrane fatty acids of *E. affinus*, to tracking sewage pollution within a deep-sea food web.

Sample Collection

Two pairs of *E. affinus* individuals were obtained from sites affected by sewage sludge dumping (Figure 1). Further samples would have facilitated a more complete study; however, the restrictions of deep-sea sampling limited the sample availability. *E. affinus* individuals were collected using the manipulator arm of the submersible DSV *Alvin* and were dissected on board the RV *Atlantis II*. Samples were stored at −20 °C. Two urchins (93−8 and 93−9) were collected in August 1992 at a depth of 2615 m, immediately west of DWD 106 where the greatest seabed loading of sewage sludge-derived organic matter was predicted (Sludge Max, 38°48.853′ N, 72°08.057′ W; *5, 6, 16*). Another two individuals (93-1 and 93-4) were collected in July 1993 at a depth of 2702 m, from a site approximately 18 km SSE of Sludge Max (station 13, 38°39.092′ N, 72°13.010′ W), along the axis of predicted benthic distribution of sewage sludge (*16*). Oxygen uptake data from Sayles *et al.* (*17*) together with bulk nitrogen and sulfur isotope data (Figure 2) indicate that the Sludge Max site was significantly more affected by sewage sludge than station 13.

Sample Extraction and Analysis

Freeze-dried gonad tissue was extracted using a modified Bligh/Dyer monophasic solvent system (*14, 18, 19*). Polar lipids (PL; assumed to be essentially membrane lipids) were

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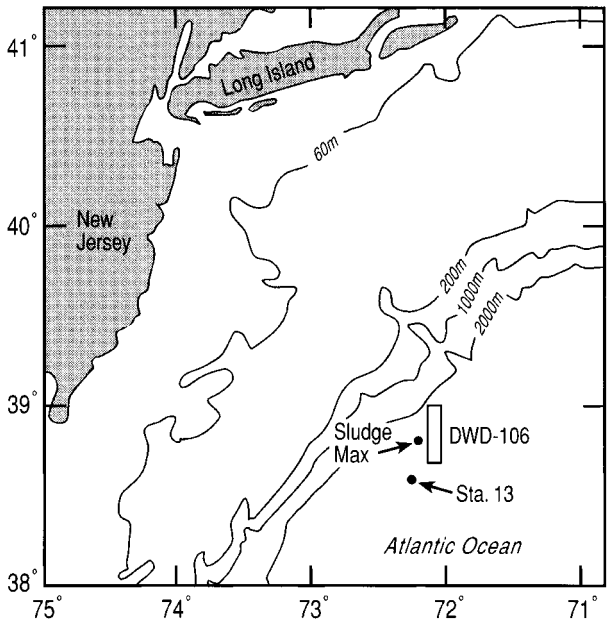

FIGURE 1. Geographical location of dumpsite DWD 106 and the deep-sea sampling locations for Sludge Max and station 13 *Echinus affinus* sea urchins.

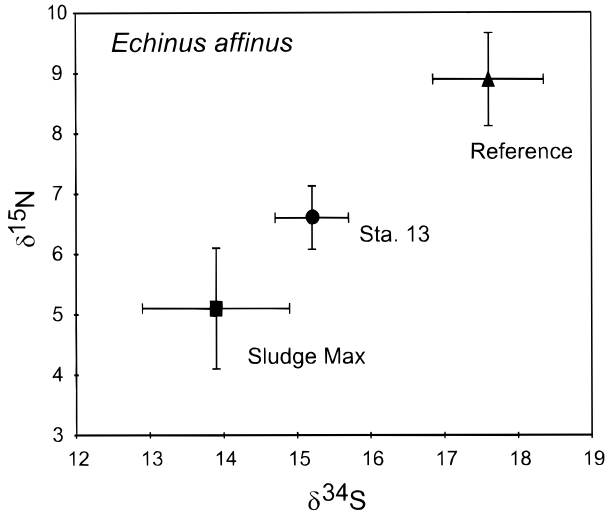

FIGURE 2. Stable sulfur isotopic compositions vs stable nitrogen isotopic compositions for *Echinus affinus* sea urchins from sites near DWD 106. Sludge Max is a site of maximum sludge input, station 13 is marginally affected. The reference site is ~100 km NE of DWD 106 and out of the influence of sludge dumping (samples from this site were not available for lipid analyses).

isolated from the total lipid extracts using aminopropyl solid phase extraction cartridges; they were then converted to their methyl esters by mild alkaline methanolysis (*14*). Polar lipids were chosen as they have a structural as opposed to a storage function, in addition to a rapid turnover rate and are thus likely to give a 'snapshot' of the diet of the urchins prior to capture.

PL fatty acid methyl esters were analyzed by gas chromatography (GC), GC- mass spectrometry, and GC−combustion−isotope ratio mass spectrometry (GC/C/IRMS) as described previously (*14*). Identification of fatty acid isomers was undertaken using mass spectral interpretation (in EI and CI modes) and equivalent chain lengths (*20*) upon capillary GC columns of differing polarity (CPSil 5CB, Chrompack, 50 m length, 0.12 μm film; OmegaWax 320, Supelco, 30 m length, 0.4 μm film). Temperature program in both cases was 40−120 °C at 12 °C min^{-1} to 250 °C at 4 °C min^{-1}, isothermal for 20 min (Figures 3 and 4). In addition, positions of unsat-

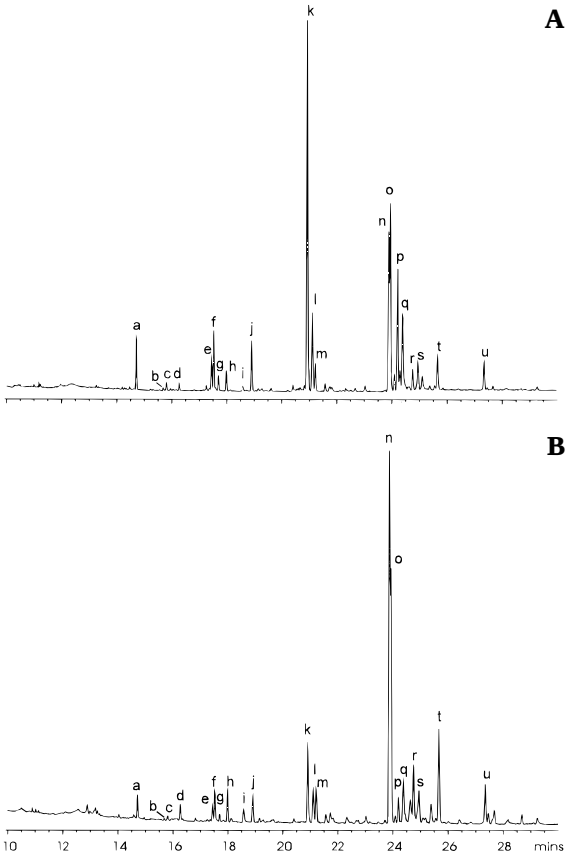

FIGURE 3. Capillary gas chromatograms of the polar lipid fatty acids (as methyl esters on dimethylsiloxane phase) isolated from *Echinus affinus* gonad tissue from (A) a site heavily affected by sewage sludge dumping (Sludge Max; 93−9) and (B) a site marginally affected by sewage sludge dumping (station 13; 93-1). Letters above peaks refer to those given in Tables 1 and 2.

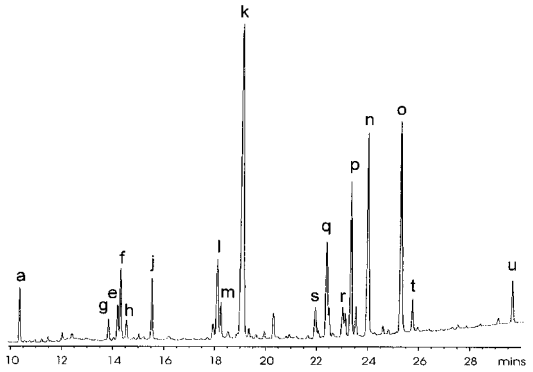

FIGURE 4. GC/C/IRMS ion-44 current of the polar lipid fatty acids (as methyl esters on OmegaWax 320 capillary GC column) isolated from *Echinus affinus* gonad tissue from Sludge Max (93-9). Letters above peaks refer to those given in Tables 1 and 2.

uration were determined by reaction of total fatty acid extracts to form dimethyl disulfide adducts (*21*) and pyrrolidide derivatives (*22*) followed by mass spectral interpretation. The fatty acid nomenclature used in the present work follows the current IUPAC−IUB convention.

GC/C/IRMS analyses were undertaken upon the OmegaWax 320 column (Figure 4). Isotopic compositions are given in the δ notation, where $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000‰$, R being the ratio of the heavier to the lighter isotope.

Results

Polar lipid fatty acid (PLFA) abundances are given in Table 1. The distributions for all the *Echinus affinus* samples

TABLE 1. Polar Lipid Fatty Acid Abundances, as Percent of Total Identified, from *Echinus affinus* Gonad Tissue

	station 13			Sludge Max			peak
fatty acid	93-1	93-4	mean	93-8	93-9	mean	id[a]
14:0	1.4	2.4	1.9	1.0	2.3	1.5	a
15:0	1.1	1.2	1.2	0	0.4	0.2	d
16:0	2.0	0	1.0	2.3	1.1	1.7	h
17:0	0	0	0	0.1	0	0.1	
18:0	0	0	0	1.5	0.5	1.0	
19:0	0	0	0	0.2	0	0.1	
20:0	0.6	1.8	1.2	0.5	0.4	0.5	
i15:0	0.1	0.4	0.3	0.2	0.1	0.2	b
a15:0	0.3	0.9	0.6	0.3	0.4	0.4	c
i17:0	0	0.7	0.4	0.8	0	0.4	
a17:0	0	0.3	0.2	0.4	0	0.2	
16:1(*n*-10)	0	0	0	0.2	0.2	0.2	
16:1(*n*-9)	1.2	2.0	1.6	1.8	2.0	1.9	e
16:1(*n*-7)	2.0	4.1	3.1	1.9	3.3	2.6	f
16:1(*n*-5)	0.5	1.0	0.8	0.4	0.8	0.6	g
17:1(*n*-9)	1.8	0	0.9	2.2	3.0	2.6	j
18:1(*n*-9)	2.8	1.9	2.4	7.0	5.3	6.2	l
18:1(*n*-7)	2.7	1.6	2.2	1.6	1.9	1.8	m
18:1(*n*-5)	0	0.3	0.2	0.3	0.5	0.4	
19:1(*n*-9)	0.7	0	0.4	1.1	0	0.6	
20:1(*n*-13)	0.9	0	0.5	2.3	0	1.2	
20:1(*n*-9)	3.0	2.6	2.8	5.1	3.0	4.1	s
20:1(*n*-7)	1.3	0	0.7	0.7	0	0.4	
22:1(*n*-9)	0.8	2.4	1.6	0.8	0	0.4	
br17:1	1.2	0	0.6	0.4	0.4	0.4	i
16:2(*n*-6)	0	0	0	0.2	0.3	0.3	
18:2	0	0	0	0.2	0.4	0.3	
18:2(*n*-6)	5.9	4.3	5.1	14.1	25.4	19.8	k
20:2Δ5,11	3.6	2.9	3.3	11.5	5.5	8.5	q
20:2Δ5,13	0.7	0.8	0.8	1.6	0.8	1.2	
20:2(*n*-12)	0	0	0	0.8	0.3	0.6	
20:2(*n*-6)	3.1	10.6	6.9	4.6	1.7	3.2	r
22:2	0.2	0	0.1	1.0	0	0.5	
22:2	0.6	0	0.3	0.2	0	0.1	
20:3	0.6	0.9	0.8	2.2	1.2	1.7	
20:3(*n*-9)	2.1	2.2	2.2	8.2	8.6	8.4	p
20:3	0.1	0	0.1	0.8	1.5	1.2	
20:4(*n*-6)	31.0	16.7	23.9	12.9	12.3	12.6	n
21:4(*n*-6)	8.0	5.0	6.5	1.9	2.6	2.3	t
20:5(*n*-3)	16.2	31.1	23.7	5.1	11.7	8.4	o
22:6(*n*-3)	3.5	1.9	2.7	1.6	2.1	1.9	u
unsat index[b]	314	316	315	222	254	238	
ACL[c]	19.5	19.4	19.5	19.1	18.8	19.0	

[a] Letters refer to peaks indicated in Figure 3. [b] Unsaturation index, concentration of unsaturated acid × number of unsaturated bonds. [c] Average chain length, $\sum C_{no} c_i$ where c_i is the concentration of the acid containing C_{no} carbon atoms.

analyzed are characterized by a wide variety of isomers as may be expected for the PLFA of benthic scavengers (*11*). Variation in the fatty acid abundances between the pairs of samples from the two sites examined is likely to be caused by a degree of natural variation (Table 1). However, clear abundance differences distinguish the samples collected from heavily polluted (Sludge Max) and marginally polluted sites (station 13; Table 1; Figures 5 and 6). Therefore, the mean abundances of the sample pairs were calculated and used throughout this study (Table 1).

E. affinus samples from Sludge Max are characterized by higher abundances of di-unsaturated fatty acids such as 18:2(*n*-6) and 20:2Δ5,11 as compared to samples collected from station 13 (Table 1; Figures 5 and 6). In contrast, the highly unsaturated fatty acids 20:4(*n*-6) and 20:5(*n*-3) are relatively more abundant in the station 13 samples than in samples from Sludge Max (Table 1; Figures 5 and 6). The overall differences between the fatty acid distributions are further illustrated by the unsaturation index (a measure of

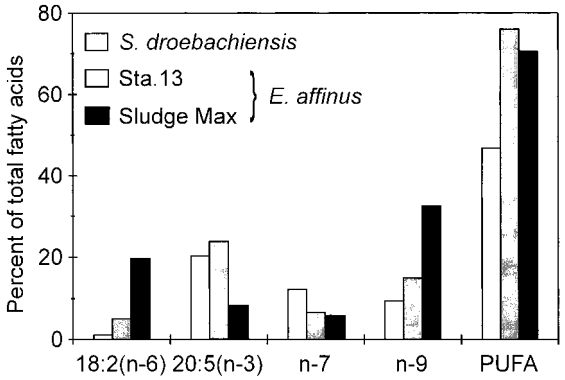

FIGURE 5. Histogram of the relative abundances (% total identified) for the deep-sea sea urchin *Echinus affinus* gonad tissue polar lipid fatty acids from a site heavily affected by sewage sludge dumping (Sludge Max; 93-9), from a site marginally affected by sewage sludge dumping (station 13; 93-1), and from the coastal sea urchin *Strongylocentrotus droebachiensis* (data from ref 19). Data given for the individual fatty acids 18:2(*n*-6) and 20:5(*n*-3). *n*-7 and *n*-9, fatty acid classes linked by the position of terminal unsaturation. PUFA = total polyunsaturated fatty acids.

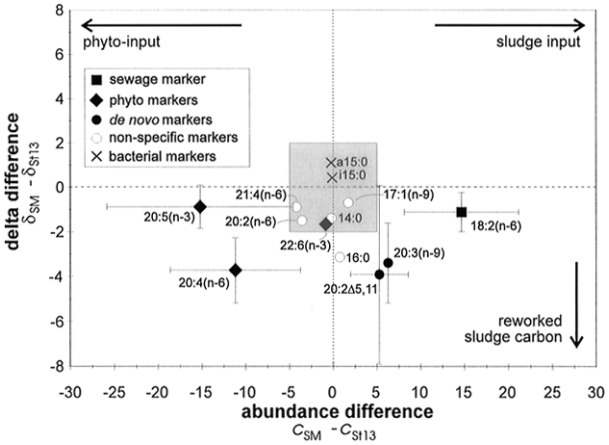

FIGURE 6. Mean abundance differences against mean δ^{13}C value differences for *Echinus affinus* fatty acids (for those acids given more than three asterixes in Table 2). Differences are calculated for Sludge Max minus station 13 values (see Tables 1 and 2). Range bars (minimum difference to maximum difference) are given for fatty acids of >5% relative abundance. The shaded area illustrates a region where differences are difficult to discern from natural variation.

overall unsaturation), which is much higher for the station 13 samples (mainly due to the difference in polyunsaturation; Figure 5), and by the average chain length, which is longer (Table 1). As a comparison, summarized data from Takagi *et al.* (*19*) on the fatty acid composition of the sea urchin *Strongylocentrotus droebachiensis* collected from the coast off Nova Scotia, are given in Figure 5.

Individual fatty acid δ^{13}C values are given in Table 2. Due to the complexity of the fatty acid methyl ester chromatograms, coelution was a significant problem in measuring δ^{13}C values for certain fatty acids (especially the mono-unsaturated fatty acids; Figure 4; also see Goodman and Brenna, ref 23). The GC column that gave the least problems with coelution was the polar OmegaWax 320. This column shifts the retention times of unsaturated relative to saturated isomers as compared to that of the apolar methylsilicone phase (cf. Figures 3 and 4) and allowed for good separation of 20:4(*n*-6) and 20:5(*n*-3). Fatty acids that were well separated and/or very abundant are indicated by a number of asteriks (1−5; the greater the number, the greater the confidence in the δ^{13}C value) in Table 2 and plotted in Figure 6.

TABLE 2. Stable Carbon Isotopic Compositions ($\delta^{13}C$ Values) of Individual Polar Lipid Fatty Acids Isolated from *Echinus affinus* Gonad Tissue from Sites Affected by Sewage Sludge Dumping

	station 13			Sludge Max				
fatty acid	93-1	93-4	mean	93-8	93-9	mean	peak code[a]	data quality[b]
14:0	−21.4	−19.9	−20.7	−22.7	−21.4	−22.1	a	*****
15:0	−22.3	−22.7	−22.5	−24.1		−24.1	d	****
16:0	−21.0		−21.0	−24.6	−23.7	−24.2	h	****
i15:0		−24.8	−24.8	−24.4		−24.4	b	***
a15:0		−24.2	−24.2	−23.1		−23.1	c	***
16:1(*n*-9)	−21.6		−21.6		−19.2	−19.2	e	*
16:1(*n*-7)	−19.7		−19.7		−23.6	−23.6	f	*
17:1(*n*-9)	−23.5	−21.9	−22.7	−23.0	−23.8	−23.4	j	*****
18:1(*n*-9)	−19.8		−19.8		−24.4	−24.4	l	*
18:1(*n*-7)	−21.7		−21.7		−24.9	−24.9	m	*
20:1(*n*-9)	−16.1		−16.1		−19.7	−19.7	s	***
18:2(*n*-6)	−25.8	−25.9	−25.9	−27.8	−26.2	−27.0	k	*****
20:2Δ5,11	−16.6	−21.7	−19.2	−24.9	−21.6	−23.3	q	***
20:2(*n*-6)	−22.3	−21.7	−22.0		−23.5	−23.5	r	*
20:3(*n*-9)	−23.4	−25.2	−24.3	−28.6	−26.8	−27.7	p	****
20:4(*n*-6)	−20.2	−21.4	−20.8	−25.4	−23.7	−24.6	n	*****
21:4(*n*-6)	−20.1	−21.3	−21.1		−21.6	−21.6	t	*****
20:5(*n*-3)	−20.7	−22.1	−21.4	−22.0	−22.6	−22.3	o	*****
22:6(*n*-3)	−19.3	−20.4	−19.9	−21.3	−21.7	−21.5	u	*****
∑16:1	−22.9	−20.2	−21.6	−23.1	−22.1	−22.6		****
∑18:1		−21.3	−21.3	−24.7	−24.0	−24.4		****
∑20:1		−20.7	−20.7	−23.4	−21.6	−22.5		****

[a] Letters refer to those given in Figure 3. Integrated values for all mono-unsaturated isomers of chain lengths 16, 18, and 20 are also given due to significant coelution. [b] Estimate of data quality (* poor quality, ***** excellent quality); greater peak separation and/or larger peaks will generally lead to better quality $\delta^{13}C$ value in a complex chromatogram.

In general, fatty acids isolated from *E. affinus* samples from Sludge Max are more depleted in ^{13}C than those from station 13 (Table 2; Figure 6), on average having $\delta^{13}C$ vaues 2‰ more negative than corresponding station 13 fatty acids (Table 2). The greatest differences being observed for 20:2Δ5,11, 20:4(*n*-6), 20:3(*n*-9), and 16:0 (Figure 6). The most ^{13}C-depleted fatty acid was 18:2(*n*-6) (−25.8 to −27.8 ‰; Table 2).

Discussion

Both the relative abundances and carbon isotopic compositions of the polar lipid fatty acids are quite different for *E. affinus* individuals collected from the site immediately west of DWD 106 (Sludge Max) and from the site marginally affected by sludge (station 13). This implies that the dietary intake of the individuals at these two sites has been markedly different. The seasonal nature of phytoplankton blooms in the open ocean is likely to strongly affect food sources and feeding strategies of benthos on the deep-sea floor. Therefore, the background of detritus deriving ultimately from a phytoplankton source available to *E. affinus* will vary significantly throughout the year, with short periods of plentiful food and others with little. Such an effect is likely to be minimized in the present study due to the collection of all samples in midsummer (August and July, respectively, for the Sludge Max and station 13 samples). Therefore, the differences observed in the *E. affinus* samples from the two sites are most likely to derive from differences in the relative importance of four different sources in the urchin diet: phytodetritus (including reworked matter by zooplankton), sewage, microorganisms, and the reworking of dietary carbon (i.e., *de novo* biosynthesis or modification of dietary fatty acids) by the urchin. Though the factors affecting fatty acid abundances in animals are complex and dependent upon varied interactions between dietary inputs of specific fatty acids and internal metabolic activities, their relative abundance and isotopic composition can still help to determine the relative importance of specific dietary sources. In the sites under study, particular fatty acids can be used as indicators for each of these potential sources, as follows;

Phytoplankton. The polyunsaturated fatty acids 20:5(*n*-3) and 22:6(*n*-3) are major constituents of marine algae (*11, 19*). They are essential to most animals and therefore tend to be preferentially removed from diet by marine invertebrates and used within their cellular membranes (*24, 25*). These acids are likely to be indicators of a source ultimately from marine phytoplankton in *E. affinus* due to their low abundance (below 2% of total fatty acids) in sewage sludge (*26*). The greater abundance of such polyunsaturated acids in the urchin samples from station 13 compared to Sludge Max (Table 1; Figure 6) implies that the relative contribution of marine phytodetritus to the available food source is less at Sludge Max than at station 13, or, in other words, that sewage-derived carbon is of greater dietary importance in *E. affinus* at the former site.

Of the fatty acids discussed above, 20:5(*n*-3) has the greatest relative abundance difference and the least average isotopic difference between Sludge Max and station 13 samples (Table 1; Figure 6). In addition, the relative abundance of 20:5(*n*-3) in the station 13 samples is much closer to that of the coastal sea urchin *Strongylocentrotus droebachiensis* (*19*) than that of the Sludge Max samples (Figure 5). These observations together with the fact that this acid has relatively ^{13}C-enriched $\delta^{13}C$ values (Table 2) suggest that it is a useful indicator for the incorporation from diet of detritus deriving ultimately from a phytoplankton source.

Sewage. The di-unsaturated fatty acid 18:2(*n*-6) is generally of low abundance in marine invertebrates, ranging between 1 and 5% of total fatty acids (*11, 19*; Figure 5) reflecting the low abundance of 18:2(*n*-6) in most marine algae (e.g., refs 24 and 25). However, this fatty acid is a major constituent of municipal sewage sludge (up to 10% of total fatty acids; *26*−*28*) and has been shown to persist into riverine and estuarine sediments (*28*). High abundances of this fatty acid in urchins from the Sludge Max site (14.1−25.4% of total fatty acids; Table 1) suggest direct incorporation of sewage-derived

18:2(*n*-6) into the cellular membranes of these animals. This hypothesis is reinforced by the lower abundance of this acid both in the station 13 individuals and in *S. droebachiensis* (Figure 5).

Previous work has indicated that the bulk isotopic composition of municipal sewage sludge from the northeast United States is more ^{13}C-depleted than marine phytoplankton in this region (*10*). The observation that the carbon isotopic compositions ($\delta^{13}C$ values) of most of the individual membrane fatty acids are more ^{13}C-depleted in urchins from Sludge Max than from station 13 (Table 2; Figure 6) provides evidence that Sludge Max urchins derive a significant input of sewage-derived organic carbon from their diet. It is also likely that those fatty acids with the most ^{13}C-depleted isotopic compositions derive ultimately from sewage sludge. Indeed, 18:2(*n*-6), is the most ^{13}C-depleted fatty acid isolated from the urchin samples (−25.8 to −27.8 ‰; Table 2). The minimal difference in the isotopic composition of this fatty acid between Sludge Max and station 13 samples ($\Delta\delta^{13}C = 1.2$‰; Figure 6), implies that the 18:2(*n*-6) incorporated by station 13 urchins can be attributed mainly to the same source as that in the Sludge Max urchins, i.e., sewage sludge (including a contribution by eukaryotic microorganisms both prior and subsequent to dumping). This hypothesis is reinforced by the low abundance of this acid reported for *S. droebachiensis* in a study where this organism was unlikely to have ingested significant amounts of sewage-derived matter (*19*; Figure 5). In addition, 18:2(*n*-6) is much more depleted in ^{13}C than any of the fatty acids abundant in marine phytoplankton (20:5(*n*-3), −20.7 to −22.6‰; 22:6(*n*-3), −19.3 to −21.7‰; Table 2), providing *prima facie* evidence for 18:2(*n*-6) being an indicator of the relative dietary importance of municipal sewage-derived carbon to *E. affinus*. The ^{13}C-depletion of 18:2(*n*-6) combined with the high abundance of this acid in sewage (and Sludge Max *E. affinus*) indicates that this acid could provide a sensitive record of the incorporation of sewage-derived carbon into marine food webs.

With regard to monitoring sewage-affected marine environments, the ratio of the abundance of benthic invertebrate 20:5(*n*-3) and 18:2(*n*-6) polar lipid fatty acids could provide a simple and sensitive measure of the recovery of these ecosystems after the cessation of dumping. The average ratios for the Sludge Max and station 13 samples are 2.36 and 0.22, respectively. The corresponding ratio for polar lipid fatty acids from *S. droebachiensis* is 0.06 (*19*).

In addition to differences in individual fatty acid abundances in the samples examined, there are also differences in the abundances of different lipid classes. For example, the Sludge Max samples are characterized by much greater concentrations of *n*-9 fatty acids and consequently lower abundances of *n*-7 fatty acids, than are those from station 13. This is likely to be due to the high concentrations of *n*-9 fatty acids in sewage sludge (up to 25% of total fatty acids; *26, 27*) as compared to that in phytodetritus (cf. the data for *S. droebachiensis*; Figure 5).

***De Novo* Biosynthesis.** The 20:2Δ5,11 and the 20:3(*n*-9) fatty acids, which are, on average, highly depleted in ^{13}C in the Sludge Max samples as compared to those from station 13 (Table 2; Figure 6), are not observed in the fatty acids of sewage sludge (*26, 27*) and are not common in phytoplankton (*24, 25*). However, these two acids are more abundant in the Sludge Max than the station 13 urchins (Figure 6) and may derive to a significant extent from *de novo* biosynthesis by the urchins. For example, either from starting products or reworking of sewage-derived fatty acids (from 18:1(*n*-9), which is abundant in sewage sludge) rather than from direct assimilation from the diet. If this hypothesis is correct, the isotopic composition of these acids is likely to be closely related to the average isotopic composition of their diet. It follows that, the isotopic difference of 20:2Δ5,11 and 20:3(*n*-9) in urchin samples from sewage affected sites, compared to those from unaffected sites, may provide another measure of the recovery of benthic ecosystems after sludge dumping in addition to the previously proposed ratio of 18:2(*n*-6) and 20(*n*-3).

Arachidonic acid, 20:4(*n*-6), tends to be of high abundance in marine invertebrates and is more abundant in the station 13 urchins than in the Sludge Max urchins. However, this acid is also relatively highly ^{13}C-depleted in the Sludge Max relative to the station 13 samples (Figure 6). This may be evidence for a contribution of 'reworked' sludge carbon to the pool of 20:4(*n*-6), i.e. that a certain portion derives from a non-phytoplankton source in the Sludge Max urchins. One potential source may be from elongation and desaturation of sludge-derived 18:2(*n*-6), which as discussed previously is abundant in the Sludge Max samples and has a highly ^{13}C-depleted signature (Tables 1 and 2; Figure 6).

Bacteria. Bacterial input into sediments is often marked by branched fatty acids, such as i15:0 and i17:0 (*29*). Such branched fatty acids are of low abundance in all the *E. affinus* samples examined (from 0.4 to 2.3%) and exhibit only slight differences in the samples from the two sites examined (Table 1), indicating that specific bacterial markers do not have a major role in the cellular membranes of the sea urchin under study. In contrast to the majority of the fatty acids analyzed, the branched fatty acids i15:0 and a15:0 are more ^{13}C-enriched in the Sludge Max samples as compared to those from the station 13 samples (Table 2; Figure 6). Such small differences (less than 1.1 ‰) may be simply due to natural variation.

Nonspecific Fatty Acids. The majority of the remaining fatty acids for which the isotopic data were sufficiently robust (Table 2) fall in a region where differences are difficult to distinguish from natural 'environmental' variation to be expected for the parameters examined in this study. The acids that fall within this region (illustrated in Figure 6) therefore could derive from a combination of a sewage, a phytoplankton, a bacterial, or a *de novo* biosynthetic source. Therefore, these particular acids have much less potential for tracking sewage-derived organic matter in the deep-sea ecosystem under study than the fatty acids discussed previously.

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