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Abstract.—Meiofauna were sampled from the NOAA ship *Gordon Gunter* during Fall 2012 off the coast of Louisiana. At five locations near the Deepwater Horizon drilling site (located 54–115 km away) box core samples and Shipek® grab samples were collected for subsurface meiofauna and sediment analysis. The goals of this study were to: 1) perform a taxonomic analysis of the meiofauna groups Nematoda and Copepoda, 2) perform statistical analyses of animal densities and sediment characteristics, and 3) compare sampling results using two different collection devices. Nematodes were the most abundant animals recovered, ranging from 88–791 animals per 10 cm^2 area. Nematodes were represented by 60 genera in 23 families. The nematode community was dominated by one genus at the deepest location. Cluster analysis showed that there were two major groups for the five sites; nematodes from two shallow sites 81 and 84, and those from two shallow sites 85 and 86 along with deep site 82. Copepods were represented by 35 species from six families, with no animals identified at the deepest location (site 82). Cluster analysis also demonstrated two major groups similar to the two nematode groups but without site 82 present. Spearman correlation analysis revealed positive correlations among nematode, copepod, polychaete, and kinorhynch densities, and no correlations among the meiofauna densities and sediment chemistry values (metals and polycyclic aromatic hydrocarbon [PAH] concentrations). Nickel concentrations varied from 3.1–30.0 mg/kg, vanadium from 5.5–71.6 mg/kg, and PAHs from 94–395 ppb. Statistical comparison (Mann-Whitney U-test) of the box corer and Shipek® sampling equipment, using animal abundance, heavy metal analysis, and PAH data, revealed no difference between the two samplers.

Keywords: biodiversity, Copepoda, heavy metals, meiofauna, Nematoda, Shipek® grab

Many studies documenting the abundance and/or diversity of various meiofaunal animals of the Gulf of Mexico have been published recently (Montagna & Harper 1996, Escobar et al. 1997, Baguley et al. 2006a, b; Escobar-Briones et al. 2008, Landers et al. 2012, Sharma et al. 2012). Meiofauna are defined as those sediment-dwelling animals that pass through a 500 or 333 μm sieve but are retained by a 45–63 μm sieve and is a group that includes nematodes, benthic copepods, polychaetes,

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kinorhynchs, and other animals of similar sizes (Giere 2009). Recent studies documented changes in the shoreline meiofauna and microbial community following the Deepwater Horizon oil spill (DHOS) (Bik et al. 2012, Ortmann et al. 2012). The present study is aimed at analyzing the meiofauna along the Gulf continental shelf, near the DHOS and at shallow locations (65–250 m depth) not typically analyzed in meiofauna studies. The study site approximately followed the 100–200 m depth contour off the coast of Louisiana on board the NOAA ship *Gordon Gunter* in 2012, similar to previous research by our laboratory (Landers et al. 2012). We report on nematode and copepod biodiversity from the Louisiana coast near the DHOS and on the sediment chemistry relevant to possible pollution effects.

Materials and Methods

Meiofauna collection.—Sediment was collected on the NOAA ship *Gordon Gunter* during October 2012, using a Shipek® grab and a box corer (Wildco©) at five locations near the DHOS site (Fig. 1). The Shipek® grab collected sediment to a depth of 10.2 cm and the box corer collected sediment to a depth of 23 cm. From each device, the subsurface meiofauna were recovered by subcoring the sample in triplicate to a depth of 5 cm, resulting in 30 subcores collected for this report. The remaining sediment from each grab device was analyzed for metal and polycyclic aromatic hydrocarbon (PAH) levels.

Each of the five locations was sampled twice (once with each sampling device). Sample water depth varied from 65.7–250.7 m; bottom temperature varied from 18.6–22.1°C, and bottom salinity varied from 31.3–36.5 psu. Collections were made during the annual small pelagics fish survey conducted by the National Marine Fisheries Service laboratory at Pascagoula, Mississippi.

Sediment samples for meiofaunal animals were fixed in 10% formalin (5% final concentration) and later sieved (333 μm pre-sieve, followed by a 63 μm and a 45 μm final sieve) before concentrating the animals by Ludox® separation (Burgess 2001, Baguley et al. 2006a). The animals were stained with rose bengal and counted, using a counting wheel under a stereo microscope. Animal groups were identified using Higgins & Thiel (1988) and Giere (2009).

Nematode and copepod identification.—Nematodes and copepods were identified from the five box core sites only (63 μm sieve), due to time and monetary constraints. Nematodes were mounted in anhydrous glycerin and identified to genus using standard identification guides (Platt & Warwick 1983, 1988; Warwick et al. 1998). The first ~100 specimens from each of the five sites were identified and categorized to general feeding group classification according to Wieser (1953). Those feeding groups are: 1a, selective deposit feeders; 1b, non-selective deposit feeders; 2a, episubstrate feeders; 2b, scavengers/predators. Harpacticoids were cleared in lactic acid and mounted whole or dissected in polyvinyl lactophenol for identification to genus or species level (Lang 1948, 1965; Huys et al. 1996).

Heavy-metal analysis.—Sediments for metal and PAH analysis were either frozen or placed in a refrigerator on the ship. Two methods for trace metal determination were used: 1) Samples were digested at room temperature using nitric acid, and further oxidized with hydrogen peroxide. The digested samples were diluted with sulfuric acid and submitted to Southern Environmental Testing, Inc. (Florence, Alabama) for ICP analysis using USEPA *Methods for Chemical Analysis of Water and Wastes*, EPA 600/4-79-020, revised May 1994. All specimens were run in batches that included blanks (reagent and

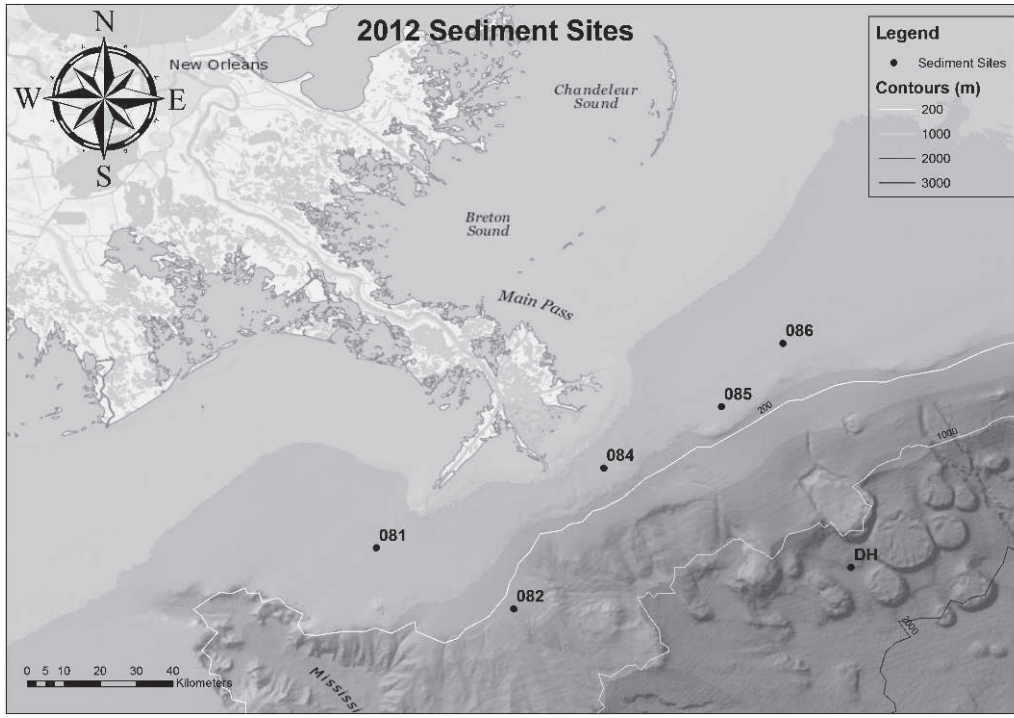

Fig. 1. Collection sites on continental shelf near the Deepwater Horizon (DH) oil spill site.

instrument) and spiked samples, and 2) sediment samples were analyzed by the Central Analytical Instruments Research Laboratory (Louisiana State University, Baton Rouge) using EPA ICP method 200.7, with all samples blank-corrected, and using nitric acid and hydrochloric acid at temperatures >100°C.

PAH analysis.—PAH concentrations in the sediments were measured by using Soxhlet extraction with methylene chloride (CH_2Cl_2) according to EPA method 3540C. Concentrations of PAHs in the extracts were analyzed by using gas chromatograph/mass spectrometry (Shimadzu GC [GC-2010] with MS [GCMS–QP2010S]).

Statistical analysis.—All numerical data were analyzed using SPSS 11.0® software. Cross-correlation analysis was used to examine the 10 site means. Site means for animal abundance were calculated from three subcores from each grab. Due to lack of normality and homogeneity of variances, the non-parametric Mann-Whitney U-test was used to examine differences in Shipek® and box core data in all categories. Analysis of site similarity was done using cluster analysis (group average, presence/absence transformation, Bray Curtis similarity index) performed on the site/species matrices using Primer 6 version 6.1.15 (Plymouth Marine Laboratories, UK).

Results

Nematode and copepod diversity.—A total of 512 nematodes were identified from the five box core samples, using the 63 μm-sieve catch (Table 1). Sixty genera from 23 families were identified. The most distinct location was site 82, the deep site,

Table 1.—Nematode diversity at 5 box core sites, using 63-μm sieved specimens. Data represents first 98–108 animals identified from each sample. * = genera not listed in recent checklists for the Gulf of Mexico (see Discussion).

Family	Genera	Feeding group	Site 81	Site 82	Site 84	Site 85	Site 86
Axonolaimidae	*Odontophora*	1b		48		1	
	Axonolaimus	1b		1			
	Synodontium	1b					1
Ceramonematidae	*Pselionema*	1a	1			1	1
Chromadoridae	*Chromadora*	2a	5		2		
	Chromadorita	2a	2				
	Chromadorina	2a		1		1	2
	Dichromadora	2a				3	3
	Neochromadora	2a	3		2	2	3
	*Parachromadorita**	2a	1				
	*Spiliphera**	2a	5				
	Actinonema	2a					1
Comesomatidae	*Dorylaimopsis*	2a	24		1	4	5
	*Hopperia**	2b			5		
	*Paramesonchium**	2b			1	1	
	Sabatieria	1b	4				
	Cervonema	1a				1	
	Comesoma	1b				4	
	Laimella	1a		1			1
Cyatholaimidae	*Marylynnia*	1b	2	2	19	6	3
	Paracanthonchus	2a		1	1	15	3
	*Minolaimus**	1a					1
	Longicyatholaimus	2a					1
	*Endeolophus**	2a					1
Desmodoridae	*Desmodora*	2a	1			1	7
	Molgolaimus	2a			2		
	Spirinia	1a		2		3	1
	*Pseudonchus**	2a		1			
	*Diplopeltoides**	1a		1			
Desmoscolecidae	*Desmoscolex*	1a	11	6	3	3	14
	Greeffiella	1a				5	4
	Tricoma	1a	1				
Diplopeltidae	*Campylaimus*	1a	2	2			
	*Diplopeltula**	1a			3		
Draconematidae	*Prochaetosoma*	1a				4	
Enchelidiidae	*Belbolla**	2b				1	
Enoplidae	*Enoplus*	2b	1				
Epsilonematidae	*Epsilonema*	1a				1	
Ironidae	*Thallassironus*	2a	1		1		
Leptolaimidae	*Camacolaimus*	2a			3	1	
	Leptolaimus	1a	1	1	2	1	
	*Antomicron**	1a					1
Linhomoeidae	*Eleutherolaimus**	1b				4	2
	Metalinhomeus	1b	1	1	17	2	
	Terschellingia	1a		12	17	12	14
	Eumorpholaimus	1b		1			
Microlaimidae	*Microlaimus*	2a	7	10	9	5	4
Monhysteridae	Genus 1	1b	24				
Oxystominidae	*Halalaimus*	1a	5	3	5	2	5
	Oxystomina	1a			1		
Pandolaimidae	*Pandolaimus*	1b					1
Selachinematidae	*Cheironchus*						2
	Halichoanolaimus	2b		1			

Table 1.—Continued.

Family	Genera	Feeding group	Site 81	Site 82	Site 84	Site 85	Site 86
Sphaerolaimidae	*Sphaerolaimus*	2b	2	5	1	6	1
Tarvaiidae	*Tarvaia*	1a				2	
Xyalidae	*Daptonema*	1b	3	1		11	15
	Gnomoxyala	1b	1		3		
	Paramonhystera	1b			1	1	
	Theristus	1b				1	1
	Scaptrella	2b			1		
Number of animals			108	101	100	105	98
Number of genera			23	20	22	30	27
Number of families			16	14	13	17	15
Number of 1a			21	28	31	35	42
Number of 2a			49	13	21	32	30
Number of 1b			35	54	40	30	23
Number of 2b			3	6	8	8	1

in which 48/101 identified animals belonged to the genus *Odontophora*, a genus recorded only once with one individual at the other four sites combined. Site 82 differed from the other sites also in the proportion of nematode feeding groups, with a higher number of feeding group 1b (non-selective deposit feeders) animals and a lower number of group 2a (episubstrate feeders) than each of the other four sites.

A total of 152 copepods (adults and copepodites) were identified from the box core sites (Table 2). Thirty-five distinct species from six families were identified. Site 82 was the most unusual in that no adult copepods were recovered from the box core sample at that site (only a single unidentified copepodite).

Analysis of site similarity.—Cluster analysis showed that there were two main groupings of the five sites for nematodes (Fig. 2A). The first group was of sites 85 and 86 (most similar) and site 82 (the deepest site). The second group included sites 81 and 84. For copepods, similar groups were revealed as for nematodes, but since there were no identified copepods found at the deepest site, this site is not among the group of four, and the four remaining sites follow the previous nematode pattern (Fig. 2B).

Comparison of sampling devices.—The box corer and Shipek® devices yielded similar results (Table 3). The Mann-Whitney U-test was used to examine differences between the two collection devices, using animal density, PAH levels, and metal concentrations, and no significant differences were obtained ($p > 0.05$). Among the 10 sediment grabs, nematodes were the dominant animals recovered in the sediment, followed by polychaetes, copepods, and kinorhynchs. Spearman correlations for all 10 collections indicated that these four animal groups were all positively correlated with each other, and kinorhynchs were negatively correlated with depth (Table 4).

Sediment chemistry and statistical correlations.—Trace metals and total PAH concentrations were analyzed to detect evidence of oil contamination in the sediment (Tables 3, 4). Method 2 (high temperature digestion) metals analysis yielded higher values than method 1, though the data from the two methods had a significant positive correlation (Ni correlation $r = 0.697$, $p < 0.05$; V correlation $r = 0.879$, $p < 0.01$). Total PAH levels and EPA priority pollutant PAH levels were very low. The PAHs analyzed (and detected, marked with *) were: *naphthalene*,

Table 2.—Copepod adults and copepodites identified at 5 box core sites, using 63-μm sieved specimens. * = not listed in recent Gulf of Mexico checklists (see Discussion).

Family	Species	Site 81	Site 82	Site 84	Site 85	Site 86
Ameiridae	Ameiridae sp. 1				3	3
	Ameiridae sp. 2					1
	Ameiridae sp. 3					1
	Pseudameira sp.*				2	2
Cletodidae	*Cletodes* sp. 1				2	
	Cletodes sp. 2				1	
	Cletodes sp. 3					5
	Cletodes sp. 4					2
	Cletodidae sp. 1					2
	Cletodidae sp. 2				1	
	Cletodidae sp. 3					5
	Kollerua sp.*	1		1	19	
	Scintis sp.*					2
Ectinosomatidae	*Bradya* sp.*				1	
	Ectinosomatidae indet.				3	2
	Halectinosoma aff. *diops**					1
	*Halectinosoma paradistinctum**				1	5
	*Halectinosoma pygmeum**					1
	Halectinosoma sp.				2	
	*Pseudobradya hirsuta**					1
	Pseudobradya sp. 1	2				4
	Pseudobradya sp. 2				1	
	Pseudobradya sp. 3				1	
Miraciidae	*Bulbamphiascus* sp.	7		1	13	10
	Robertgurneya sp. 1	2		1	11	1
	Robertgurneya sp. 2				2	
	Stenhelia (*delavalia*) sp.				4	1
	Typhlamphiascus sp.*				7	2
	Miraciidae sp. 1			1		
	Miraciidae sp. 2	1				
	Miraciidae sp. 3				1	
	Miraciidae sp. 4				1	
Normanellidae	*Normanella* sp. 1* (*mucronata*)				1	4
Paramesochridae	*Apodopsyllus* sp. 1*			2		
	Leptopsyllus sp.*				1	
Number of species		5	0	5	21	20
Number of individuals		13	0	6	78	55

2-methylnapthalene, 1-methylnapthalene, biphenyl, 2,6-dimethylnapthalene, *acenaphthylene**, *acenaphthene**, dibenzofuran, 2,3,5-trimethylnaphthalene, *fluorene**, dibenzothiophene*, *phenanthrene**, *anthracene**, carbazole*, 1-methylphenanthrene*, *fluoranthene**, *pyrene**, *chrysene**, *benzo(a)anthracene**, *benzo(b)fluoranthene*, *benzo(k)fluoranthene*, benzo(e)pyrene, *benzo(a)pyrene*, perylene, *dibenzo(a,h)anthracene*, and *benzo(g,h,i)perylene*. (EPA priority pollutant PAHs are in italics).

Metal concentrations, total PAH levels, and meiofauna data were analyzed using Spearman correlation analysis. This analysis found no correlations with Ni, V, and meiofauna abundances using either metal analysis method. Additionally, meiofaunal groups did not correlate with total PAH levels or total EPA priority pollutant PAH levels, whether analyzed using raw values or after correction for organic carbon concentration. Positive correlations were

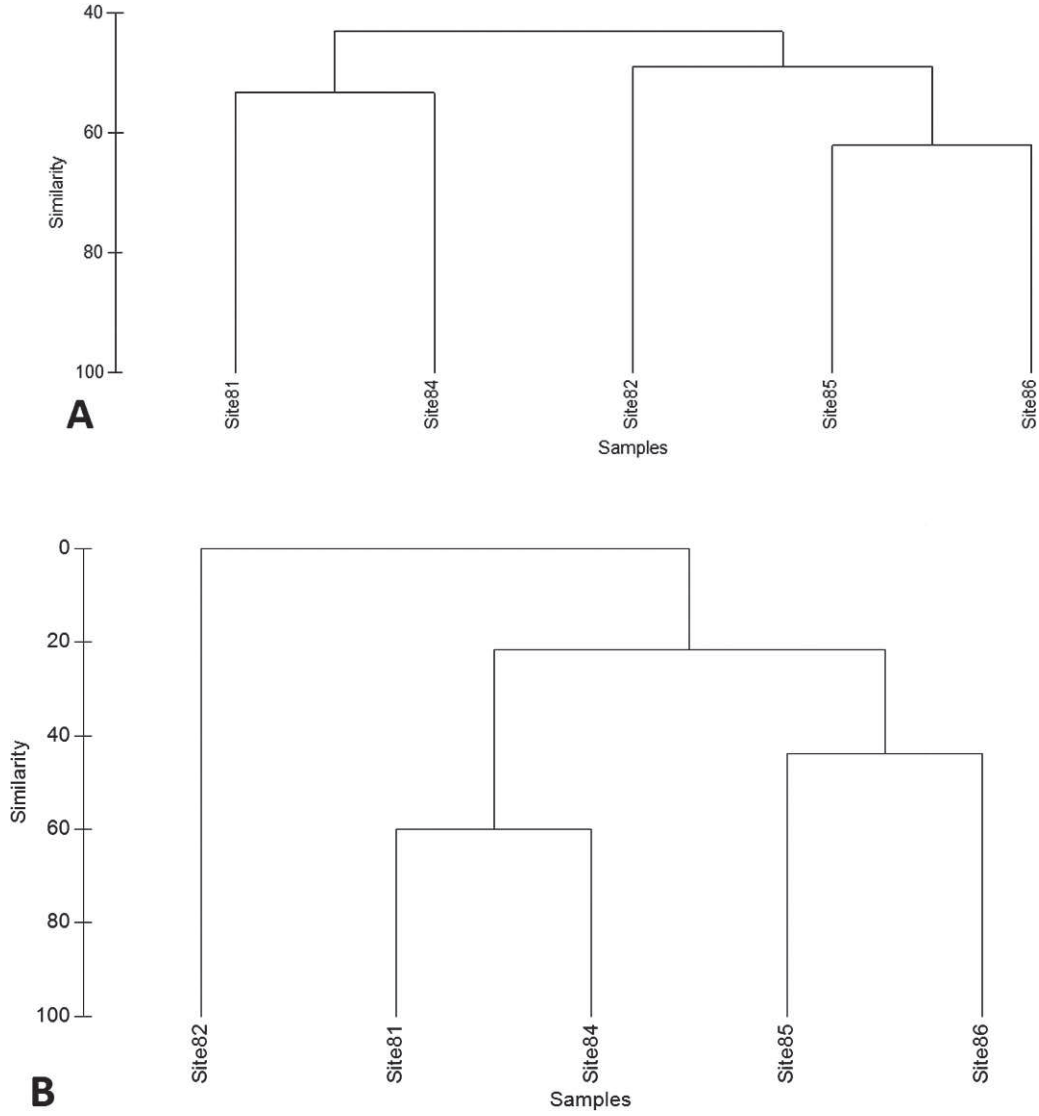

Fig. 2. Group average cluster analysis using presence/absence transformation and Bray Curtis similarity index, from 5 box core sites. A, nematode analysis; B, copepod analysis.

found between Ni and V concentrations and increasing water depth.

Discussion

This study characterized the meiofauna and sediment from sites near the Deepwater Horizon blowout of 2010. An important component of this study was to document the diversity of the nematode and copepod fauna. At this relatively unstudied area of the Gulf of Mexico shelf, we now report 60 genera of nematodes from 23 families. The deepest site (#82), while grouped with two of the shallower sites in the cluster analysis, had a different proportion of nematode feeding types with a higher proportion of non-selective deposit feeders (group 1b) than

Table 3.—Meiofauna and sediment data. Abbreviations: BC = box corer, Cop = Copepoda, Depth = water depth (m), Kino = Kinorhyncha, Lat = latitude, Long = Longitude, Nema = Nematoda, Ni = nickel in mg/kg, PAH = total polycyclic aromatic hydrocarbons in PPB, Poly = Polychaeta, Sal = salinity in psu, SG = Shipek® grab, Temp = temperature in °C, V = vanadium in mg/kg. All animal densities are the mean of 3 subcores, expressed as #/10 cm^2. Ni and V concentrations presented using method 1 and method 2 (in parentheses).

Site	Nema	Cop	Poly	Kino	Lat	Long	Depth	PAH	Ni	V	Temp	Sal
81BC	347.4	3.3	18.7	0.2	28.77928	−89.56422	88.1	395	16.23 (25.98)	20.98 (55.47)	20.94	36.50
81SG	244.7	2.0	3.7	0.4	28.78352	−89.56122	87.5	234	16.9 (26.54)	22.42 (55.60)	20.94	36.50
82BC	104.0	0.2	0.4	0.2	28.62983	−89.21851	250.7	347	17.41 (28.19)	28.60 (66.33)	18.62	36.47
82SG	88.2	1.1	1.1	0.0	28.63315	−89.2212	242	141	17.5 (30.06)	29.03 (71.68)	18.62	36.47
84BC	241.4	1.3	5.5	0.4	28.97825	−89.00217	84.9	218	17.27 (22.33)	20.63 (51.65)	21.77	31.33
84SG	791.7	21.3	5.9	4.4	28.98084	−88.99902	84.8	94	21.18 (25.20)	23.12 (55.10)	21.77	31.33
85BC	457.5	19.7	24.8	8.6	29.13122	−88.70798	87.2	174	13.13 (26.16)	20.75 (62.42)	20.99	35.70
85SG	131.9	3.1	3.3	1.1	29.13242	−88.70763	86.1	144	12.05 (21.43)	17.98 (53.41)	20.99	35.70
86BC	342.3	17.1	26.1	2.4	29.28862	−88.55649	65.7	123	3.1 (5.63)	5.52 (13.94)	22.1	35.60
86SG	162.6	2.6	10.8	0.7	29.28921	−88.55590	65.7	159	3.29 (7.14)	6.22 (16.90)	22.1	35.60

the other sites due to the dominance of the genus *Odontophora*. Thirty-five copepod species from six families were represented in our collection, with the highest diversity at sites 85 and 86 (site 84 may have also revealed high diversity had the Shipek® samples been identified). The deepest box core site had no adult copepods, which is consistent with the low density of nematodes and polychaetes at that site. Many of the organisms reported in this paper were not found in recent checklists of Gulf of Mexico nematode or harpacticoid species (indicated on Tables 1, 2 with an asterisk) (Burgess et al. 2005, Hope 2009, Suárez-Morales et al. 2009, Sharma et al. 2012).

Nematode densities that we report are higher than those reported earlier in southeastern Louisiana in 2007–2009 near the 200 m contour line (Landers et al. 2012:Fig. 2, seven sites east of Mississippi Canyon). The current study reports an average nematode density of 245 animals/10 cm^2 (63-μm sieve) and 291 animals/10 cm^2 (63 + 45-μm sieve). The seven Louisiana locations situated to the east of the Mississippi Canyon in 2007–2009 (Landers et al. 2012) had a nematode density of 143.5 animals/10 cm^2 (63-μm sieve). The density of nematodes from the current study matches closely with the results reported by Sharma et al. (2012). That study, of meiofauna collected in 2000 from the Gulf slope and deep sea (212–3000 m depths), reported nematode densities of 221/10 cm^2 (North Florida shelf) and 277/10 cm^2 (Texas shelf) at the shallow 212 m sites, using a 45-μm sieve. Nematode densities are similar to or higher than pre-oil spill levels. It is possible that the area at this depth contour along the continental shelf was not severely affected by the DHOS or that its effects were not detectable in 2012.

The deepest site had the lowest nematode and copepod densities, which may suggest that depth is an influential environmental factor. This trend of decreasing abundance with increasing depth has been documented for meiofauna in other studies (Coull et al. 1982, Pequegnat et al. 1990, Baguley et al. 2006a). Sites 85 and 86, of similar depth, had similar meiofaunal densities and grouped together on both cluster analyses, yet possessed very different levels of trace metals, suggesting that metals concentration had little influence on the meiofauna. In addition to Ni and V, those two sites (85 and 86) had marked differences in other trace metals (Al, Cu, Pb, and Zn, data not shown) with lower concentrations in the more easterly site #86. The sediment contaminants may be influenced greatly by the complex interac-

Table 4.—Spearman correlational analysis from 2012 sediment collections ($N = 10$). * = $p < 0.05$; ** = $p < 0.01$, *** = $p < 0.001$. Abbreviations follow Table 3. EPA PP = EPA priority pollutant PAHs, PAH/OC = PAHs divided by organic carbon. Ni and V correlations expressed for method 1 and method 2 (in parentheses). Longitude converted to positive values for the correlations.

Variable	Nema	Cop	Poly	Kino	Lat	Long	Depth
Cop	0.867**						
Poly	0.758*	0.770**					
Kino	0.659*	0.817**	0.604				
Lat	0.297	0.576	0.612	0.756*			
Long	−0.042	−0.370	−0.382	−0.689*			
Depth	−0.389	−0.547	−0.608	−0.685*	−0.900**	0.790**	
PAH	−0.103	−0.430	−0.164	−0.506	−0.515	0.588	0.584
PAH/OC	−0.139	−0.248	0.248	−0.207	0.103	−0.164	−0.061
EPA PP	−0.091	−0.406	−0.152	−0.470	−0.479	0.564	0.517
EPA PP/OC	−0.042	−0.067	0.430	−0.110	0.273	−0.261	−0.213
Ni	−0.079 (−0.261)	−0.297 (−0.467)	−0.588 (−0.576)	−0.384 (−0.549)	−0.745* (−0.867**)	0.612 (0.758*)	0.511 (0.900***)
V	−0.188 (−0.248)	−0.370 (−0.370)	−0.624 (−0.539)	0.518 (−0.451)	−0.867** (−0.794**)	0.733* (0.661*)	0.778** (0.900***)
Temp	0.419	0.542	0.665*	0.681*	0.886**	−0.788**	−0.988***
Sal	−0.246	−0.345	−0.295	−0.582	−0.591	0.689*	0.790**

tions between the loop current and the Mississippi river outflow (Androulidakis & Kourafalou 2013), though a more thorough analysis of multiple sites in this area will be needed to examine current effects on meiofauna and sediment characteristics.

Sediment nickel and vanadium concentrations varied from 3.1–71.6 mg/kg and total PAH levels varied from 94–395 ppb. The chronic exposure benchmark values for Ni and V sediment contamination (Ni = 20.9 mg/kg, V = 57 mg/kg) were exceeded at many of the sites (USEPA 2013). PAH levels were well below the benchmark value for sediment contamination (total PAHs lower screening value = 4022 ppb [Nowell et al. 2013]). Interestingly, our sediment contaminants were above those values for trace metals and PAHs reported from nearshore locations contaminated by the DHOS (Floyd et al. 2012, Liu et al. 2012). However, the origin of these metals and PAH concentrations could come from a number of sources, including the DHOS, the numerous drilling and ship operations along the Louisiana coast, and from runoff from the Mississippi River. Regardless of their source, no correlation between meiofaunal density and sediment contaminants was found. Meiofauna density and community structure appear to be influenced by depth.

A final goal of the study was a comparison of sampling methods, both in the field and in the laboratory. When the Shipek® grab vs. box corer samplers were compared, we found no statistical difference in the results. This suggests to us that results from these samplers can be combined and that both samplers performed equally well in collecting the subsurface meiofauna.

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