Polychaete Annelid (segmented worms) Abundance and Species Composition
in the Proximity (6 to 9 km) of the Deep Water Horizon (DWH) Oil Spill
in the Deep Gulf of Mexico

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Abstract: Polychaete annelids (segmented worms) dominated the macrobenthos in sediments located 5 to 9 km from the Deep Water Horizon (DWH) Oil Spill site five months after the event, based on nine 0.2 m² box core samples. Numbers of species, abundance, and biodiversity indices in the polychaete taxa were significantly lower than pre-spill values from similar depths in the eastern Gulf of Mexico (GoM). Both non-selective and selective deposit feeders were the most frequent feeding guilds, as expected, but their abundances were significantly lower. An increase in the number of carnivorous Sigalionidae may be a response to an accumulation of petroleum hydrocarbons on the sediment. The concentration of oil in the sediments was low and the source of the oil remains equivocal. Multivariate analyses illustrated the differences between communities near the DWH and those from prior studies in similar deep GoM habitats. In summary, Deep Water Horizon oil spill appears to have had a measurable impact on the polychaetes.

Keywords: Deep Water Horizon Oil Spill, polychaete, deep Gulf of Mexico, DGoMB, PAH.

1. Introduction

The deepwater horizon (DWH) oil spill began on April 20, 2010, in the Gulf of Mexico at ~ 1500m depth (Fig. 1.). Effects on some groups, such as vertebrates (Antonio *et al.*, 2011; Whitehead *et al.*, 2011), benthic microbial eukaryotes (Bik *et al.*, 2012), and benthic macrofauna and meiofauna (Montagna *et al.*, 2013) have been reported, but the overall initial and long-term influences of this unprecedented alteration of the deep-water ecosystem are emerging more slowly. The influence of oil on marine animals is known to vary according to the amount, concentration, duration of exposure and location (Gin *et al.*, 2001; Ingole *et al.*, 2006).

Generally, benthic animals can be affected by the toxic effects of PAHs (Polycyclic aromatic hydrocarbons) and organic enrichment (Gomez Gesteira & Dauvin 2005; Mohammad, 1974). Oil spills, fossil fuel combustion and terrigenous combustion of biomass introduce PAHs into the marine environment. The hydrophobic nature of PAHs makes them prone to deposition in marine sediment where they can be concentrated in marine invertebrates, especially deposit-feeders (Giessing *et al.*, 2003; Meador *et al.*, 1995; Soliman and Wade, 2008). The meager metabolism of PAHs and bioaccumulation in invertebrates can affect entire foodwebs (Somero, 1992). Organic enrichment often leads to proliferation of opportunistic species (Conlan *et al.*, 2004), but different taxonomic groups can have a variety of positive and negative responses to oil. The numerically dominant polychaete annelids (segmented worms) appear to be among the most responsive among all the sea floor taxa and therefore have been adopted as pollution indicators (Dean, 2008; Gomez Gesteira and Dauvin, 2000). If oil were deposited on the sea floor around the DWH event site (White *et al.*, 2012), then this contamination might be reflected in an alteration of functional feeding types, as well as species composition.

Montagna *et al.* (2013) were able to define a benthic footprint around the Deep Water Horizon blowout using the ratio of nematode worms to harpacticoid copepod crustaceans in multicores. Changes in species composition were not documented.

Benthic community distributions in the deep Gulf of Mexico are fairly well-known. For example, the biomass, abundance, species composition, and biodiversity can be predicted as a function of location and depth (Rowe, Polloni, and Horner, 1974; Pequegnat, Gallaway and Pequegnat, 1990; Wang, 2004; Baguley *et al.* 2006a and b, 2008; Sharma *et al.* 2011; Wei *et al.*, 2010, 2012a and b; Carvalho *et al.* 2013). We thus hypothesized that the sediment infaunal species composition, abundance and biodiversity would be useful indicators of the impact of and eventual recovery from the spill by comparing the ‘new’ post-spill fauna with the extensive historical database. Thus, the aims of the current paper are to 1) present the species composition, feeding type, and community structure of deep-sea polychaete species in close proximity (5 to 9 km) to the Deep Water Horizon oil spill site and 2) compare the post-spill species composition with the extensive information in the large pre-spill database, particularly within the dominant polychaete worm assemblages.

2. Materials and methods

2.1. Sampling methods

DWH samples were obtained on Sept. 25, 2010, five months after the oil spill began and thus two months after it was terminated, during a research mission of the Motor Yacht **Arctic Sunrise**. Sampling locations were opportunistic and based solely on proximity to the DWH event site and available wire time during the mission. Three locations were sampled within 5 to 9 km from the well head (Numbers 15, 17, 18; Fig.1a-b) at the following locations: 28.70N x

88.44W; 28.83Nx88.34W; and 28.79N x 88.29W, respectively. These locations were within the “moderately impacted area” of the footprint defined by Montagna *et al.*(2013).Three replicates were taken at each site for a total of 9 samples. Their depth ranged from 1189 to 1397 m. The samples were taken with a GOMEX box corer (Boland and Rowe, 1991) that covered an area of 0.2 m², but removal of subsamples reduced the total area to 0.18 m². The subsamples were taken with polycarbonate tubes; the total oil equivalents (TOE) and the PAH data presented in this paper were measured on a single sub-core from each core. The total area of the sub-samples amounted to 0.02 m². The surface 15 cm of sediments in each core, minus the subsamples, were sieved at sea through 300 micrometer mesh stainless sieves and then fixed with 10% buffered formalin in filtered sea water. Then, in the lab, each of the nine samples was stained with 5% Rose Bengal for 24 hours and sorted to major taxa using a dissecting microscope. Polychaetes were further identified to species or lowest possible taxonomic level. Samples were preserved in 70% ETOH following sorting.

Four of the nine samples (15-1, 17-1, 17-2 and 18-2) were leaking sea water from the bottom jaws on recovery of the corer, and these have been noted in the Results (Table 1).

2.2. Pre-Oil Spill Historical Database

For comparison, we utilized pre-oil spill polychaete species data from nine locations sampled during the Deep Gulf of Mexico Benthos (DGoMB) study sampled in 2000 to 2002 (Rowe and Kennicutt, 2008;Wei *et al.*, 2010). These sites were all on the continental slope adjacent to the DWH region (Fig.1-a, Table 1).They were not at precisely the same location as the DWH samples, and their depth ranged from 334-1825m, with two replicates per site, except for the HiPro location with only one. Two visits were made to S36 in DeSoto Canyon: June, 2000 and

2001. Detailed information on these locations can be found in a set of papers in a special volume of Deep-Sea Research II (Rowe and Kennicutt, 2008), with additional faunal analyses presented in Wei *et al.* (2010, 2012a and b) and Carvalho *et al.* (2013). Polychaete species lists can be found in Hubbard (1995) and Wang (2004). Voucher specimens of each species identified by name and each species designated by letter are retained presently in working voucher collections at Texas A&M at Galveston.

2.3. Total Oil Equivalents (TOE) and Polycyclic Aromatic Hydrocarbons (PAH) Analysis

The top 2 cm of one sub-core from each location were used for Total Oil Equivalents (TOE) and Polycyclic Aromatic Hydrocarbons (PAH) analyses. Estimates of total oil equivalents (TOE) of DWH oil were based on the maximum intensity (MI) measured at an excitation wavelength of 260 nm and an emission wavelength of 370 nm (Wade *et al.*, 2011) corresponding to MI from a sample of DWH oil (Sample QA10OIL01 from NIST). The analysis of PAHs used the NOAA NS&T Methods (Denoux *et al.*, 1998; Qian *et al.*, 1998; Wade *et al.*, 1993). Sediment samples were oven dried at 40°C, mixed with hydromatrix and extracted with methylene chloride in an Automated Solvent Extractor (ASE). The sample extracts along with procedural blanks were then analyzed by total scanning fluorescence. Samples were diluted, when necessary, to minimize quenching effects. Sample extracts (including matrix spike and duplicate) were reduced in volume to 1 ml and separated from possible interfering compounds using silica/alumina columns. The purified extracts were analyzed on an Agilent 6890/5973 gas chromatograph with a mass selective detector (GC/MS) using a selected ion detection technique. The GC/MS was calibrated with known concentrations of analytes at five different concentration levels and the average relative response factors of the analytes were used for PAH concentration

determination. Concentrations of PAHs are reported as nanogram/gram (ng/g) on a dry weight basis for sediment samples. Both non-alkylated (parent PAHs) and alkylated PAHs are reported (Wade *et al.*, 2008).

Fig.1 (a) Locations of historical sampling and (b) Post DWH spill sampling sites at 15, 17, and 18 (see 2.1. and 2.2.).

2.4. Data Analysis

Biodiversity was estimated using Margalef's richness index (d) (Margalef, 1968), Pielou's evenness index (j') (Pielou, 1975) and the rarefaction index $Es(n)$ (Sanders, 1968), where $Es(n)$ is the expected number of species in a sample of n individuals.

A species-sample matrix was square-root or forth-root transformed before multivariate analysis. Primer 6.0 was employed to calculate d , j' and $Es(100)$. Cluster Analysis was utilized to illustrate differences in shared species between locations (the Bray-Curtis similarity measure). A Student's t-test was used to compare means. Polychaete feeding types were determined based on categories described by Fauchald and Jumars (1979). Regression plots were created by SigmaPlot 10.0.

3. Results

3.1. Polychaete Abundance, Species Composition and Alpha Diversity

In total, 1035 individual polychaetes were found in the DWH samples; they belonged to 32 families and 165 species. The average abundance was 636.0 inds/m² (Table 1). Sample 18-1 had both the highest number of species and abundance, whereas 15-2 had the lowest number of species and 18-2 had the fewest individuals.

Table 1. Number of polychaete species (S), individuals (N), abundance and diversities of DWH, along with similar information in the continental slope database (the DGoMB study). In the historical data (DGoMB), 1C1-1 refers to a station C1, replicate 1, on Cruise 1 (2000), 2-S36-2 refers station S36, replicate 2, on Cruise 2 (2001). "all" indicates that all DWH samples are included in statistical test; "remove washed" indicates analysis without the four samples that leaked sea water; d is Margalef's richness index, j' is Pielou's evenness index and $Es(n)$ is $Es(100)$ Rarefaction Index with 100 individuals. A Student's t-test was adopted to test the differences between the means of those parameters in the two sets of data. All locations are pictured in Figure 1a and b.

		Depth(m)	S	N	Abundance(inds/m ²)	d	J'	Es(100)
DWH	15-1*	1397	24	96	530.9	5.04	0.77	24
	15-2		22	42	232.3	5.62	0.93	22
	15-3		30	95	525.4	6.37	0.91	30
	17-1*	1189	41	114	630.5	8.45	0.87	38.3
	17-2*		29	50	276.5	7.16	0.95	29
	17-3		60	199	1100.5	11.15	0.85	40.3

	18-1	1318	64	306	1692.3	11.01	0.83	36.9
	18-2*		25	36	199.1	6.7	0.94	25
	18-3		39	97	536.5	8.31	0.89	39
	Mean		37	115	636	7.75	0.88	31.6
	Std.Deviation				453.12	2.06	0.06	6.72
Historical Database (DGoMB)	1C 1-1	334	47	298	1727.5	8.07	0.86	32
	1C 1-5		49	122	707.2	9.99	0.92	44.4
	1C 4-1	1455	56	209	1211.6	10.3	0.86	40.2
	1C 4-2		53	199	1153.6	9.82	0.85	38.8
	1C 7-1	1080	61	302	1750.7	10.51	0.81	38.1
	1C 7-2		71	250	1449.3	12.68	0.87	45.1
	1MT1-3	482	66	612	3547.8	10.13	0.76	32
	1MT1-4		71	474	2747.8	11.36	0.79	34.8
	1MT2-1	676	60	590	3420.3	9.25	0.8	31.1
	1MT2-4		58	499	2892.7	9.17	0.8	31.7
	1MT3-1	983	95	411	2382.6	15.62	0.82	44.4
	1MT3-2		63	379	2197.1	10.44	0.86	38
	1MT4-1	1401	65	261	1513	11.5	0.87	41.6
	1MT4-2		56	197	1142	10.41	0.88	40.4
	1S36-1	1825	87	544	3153.6	13.65	0.76	38.5
	2S36-2		62	404	2342	10.16	0.77	33.6
	2HiPro-1	1574	62	414	2400	10.12	0.79	32
	Mean		64	363	2102.3	10.78	0.83	37.5
	Std.Deviation				833.37	1.74	0.05	4.71
p	All		0	0	0	0.001	0.016	0.048
	*remove washed		0.008	0.007	0.005	0.034	0.031	0.019

All measured biological variables (number of species, abundance, and diversity) were significantly different (lower) between DWH in 2010 and the historical database (Table 1), with the exception of Evenness, j' , which was higher, meaning the individuals were more evenly distributed among the species present.

None of the dominant species in the samples was the same as those in the database (Table 2). In both sets, the most dominant species were in the family Maldanidae, but the species were

different. The *Sthenelais* sp., in the family Sigalionidae, was abundant at the DWH site, but not in the older data. While the sigalionid *Sthenelais* sp. was the 2nd most abundant post-event species, the most abundant species (*Pholoe* sp. B) of this family ranked 57th among all the species in the database. The spionids had no prominent species in the proximity of the DWH site, but were prominent in the database (Table 2).

Table 2. Dominant species of post-spill 2010 DWH and the pre-spill 2000DGoMB studies.

	Family	Species	Average Abundance(inds/m ²)
DWH	Maldanidae	<i>Micromaldan</i> esp.	73.1
	Sigalionidae	<i>Sthenelais</i> sp.	52.8
	Maldanidae	<i>Maldan</i> esp. A	45.5
	Paraonidae	<i>Aricidea</i> sp.3	39.9
	Syllidae	<i>Exogone</i> sp. 3	30.1
	Cossuridae	<i>Cossura delta</i>	29.5
Historical Database	Maldanidae	<i>Maldanida</i> esp.	179.7
	Spionidae	<i>Spionida</i> esp.	176.3
	Paraonidae	<i>Aricidea</i> suecica	101.6
	Spionidae	<i>Prionospio</i> cirrifer	73.7
	Paraonidae	<i>Aedicira</i> sp.	65.5

3.2. Feeding types

The non-selective deposit feeders, selective deposit feeders and suspension filter feeders were significantly lower after the event compared to the historical data (Table 3). The abundances of carnivores, scavengers and omnivores were not significantly different however.

The two dominant families, Capitellidae and Paraonidae, are both considered to be non-selective deposit feeders, but only the Paraonidae decreased significantly. Of the selective deposit feeders

(Cirratulidae, Maldanidae and Spionidae), only the Spionidae dropped in abundance after the spill.

The Family Sigalionidae was significantly higher in the post-event samples due largely to

Sthenelais sp. (Table 4), the only family with higher abundances in the post-event samples.

Scavengers (S) and suspension feeders (SF) were rare in both historical and DWH assemblages.

No change could be detected in the omnivorous Syllidae.

Table 3. Mean abundance (inds/m²) of each feeding type. C = Carnivore, NSDF = Non-selective deposit feeder, O = Omnivore, S = Scavenger, SDF=Selective deposit feeder and SF = Suspension filter feeding. A Student's t-test was used to test differences in means of the abundances of each feeding guild in the post-spill data and the historical database.

Survey	C	NSDF	O	S	SDF	SF
DWH	110	228	86	1.23	204	1.8
DGoMB	148.6	780.7	212.8	8.31	914.3	37.7
p	0.305	0.003	0.05	0.146	0.001	0.003

Table 4. Mean abundance (inds/m²) of dominant families. Siga. (Sigalionidae), Capi. (Capitellidae), Para. (Paraonidae), Cirra. (Cirratulidae), Sylli. (Syllidae), Mald. (Maldanidae) and Spio.(Spionidae). Student's t test used to test the null hypothesis that the relative (%) mean abundance of each dominant family were the same in pre and post event data.

Survey	Siga.	Capi.	Para.	Cirra.	Mald.	Spio.	Sylli.
DWH	57.1	86.6	86.6	30.1	121.7	32.6	64.5
DGoMB	25.9	100.2	481.5	91.7	232.2	476	127.8
p	0.007	0.735	0.003	0.063	0.269	0.011	0.134

3.3. Total Oil Equivalent (TOE) and Polycyclic Aromatic Hydrocarbons (PAH)

Total oil equivalents (TOE) ranged from 50 up to 850ug/g of sediments, while the corresponding PAH ranged from 100 up to 1000ng/g of sediment (Fig. 2, Table 5). A linear relationship between the two was highly significant.

Fig. 2.Total PAH as a function of “total oil equivalents” from subsamples of the nine cores near (5 to 9 km) the DWH well head (Fig. 1.).

Total PAH (TPAH) concentration in our samples near the footprint averaged 417.5 ± 247.1 ng/g.

Fifteen selected PAH (ng/g) in the DWH samples are listed in Table 5. The total PAH in the database (Wade *et al.* 2008) prior to the spill declined with depth (Fig.3.). Most of the post-event values were above the 95% confidence interval of the historical values at the depths sampled in this study (1.2 to 1.4 km), reinforcing other observations of DWH-derived oil and PAH in or near the ‘footprint’ of the event (Montagna *et al.* 2013). Only two of the values at the 9 sites were on or below the regression line.

Fig.3.Total PAH in the DWH samples compared to that in the historical samples (DGoMB samples in Wade *et al.* 2008) plotted as a function of depth. The linear regression (solid line) is encompassed by the 95% confidence interval (dashed lines) of the pre-event data.

Table 5. Total PAH (TPAH) and fifteen selected PAH (ng/g) in the DWH location subsamples.

		TPAH	NAPH	ACENAPL	C1FLUOR	C3FLUOR	C1PHAN	DIBEN	C1DIBEN	C1FLPY	CHRYSEN	C1CHRYS	BENKFLU	BENAPYR	DBAHANT	D26NAPH	M1PHEN
DWH	15-1	969.8	3.7	0.8	10.1	56.2	38.9	1.1	9.7	39.8	8.8	53.2	1.9	4.4	1.4	4.9	18.8
	15-2	98.9	0.5	0.3	0.8	0.0	2.0	0.3	1.0	7.3	3.7	3.6	0.9	1.1	0.5	0.2	1.3
	15-3	450.6	3.7	1.3	2.3	12.7	9.3	0.4	2.3	27.3	4.5	20.1	1.8	9.6	1.0	1.9	2.6
	17-1	408.6	3.0	0.4	3.0	21.4	12.0	0.4	3.3	31.8	4.1	20.1	0.9	4.0	0.5	1.4	7.2
	17-2	311.1	1.7	0.5	1.5	11.6	7.4	0.0	1.9	21.0	3.2	15.7	1.0	1.7	0.4	0.7	3.7
	17-3	325.0	2.4	0.7	3.0	14.4	10.1	0.3	3.3	21.0	3.4	16.3	1.6	2.1	0.7	1.1	23.0
	18-1	467.3	4.1	1.5	2.6	12.4	11.7	0.6	2.5	39.2	6.9	24.8	2.8	4.3	1.6	2.0	4.0
	18-2	201.3	2.5	0.5	1.1	6.2	2.8	0.2	0.9	13.6	3.0	9.7	1.0	13.2	0.4	0.7	2.0
	18-3	525.0	2.3	0.8	2.3	18.5	7.4	0.4	2.0	20.8	15.4	35.4	1.0	1.4	0.7	0.7	3.8
	Mean	417.5	2.7	0.8	3.0	17.1	11.3	0.4	3.0	24.6	5.9	22.1	1.4	4.6	0.8	1.5	7.4
Std.Deviation	247.1	1.1	0.4	2.8	16.0	10.9	0.3	2.6	11.0	4.1	14.7	0.6	4.1	0.4	1.4	7.9	

3.4. Multivariate Analysis Comparing Post and Pre Event Polychaete Species

The species composition with in or near the DWH oiled footprint was very different from the historical samples (Fig.4). Likewise, the abundances were higher prior to the event (Fig.5). The species encountered in our samples were remarkably similar (40 to 50%), but were much less similar with the database (< 20%) in shared species. The species grouped on the right were all at depths of 1 km or less whereas the central group sites were all at depths between 1 and 2 km.

Fig.4. Dendrogram of Bray-Curtis similarities of historical (DGoMB) and post-event (DWH) continental slope samples.

Fig.5. Whisker plots of polychaete abundances (ind./m²) of historical (DGoMB) and post-event (DWH) continental slope samples (data from Table 1).

4. Discussion

4.1. The Influence of the DWH Oil Spill on the Polychaete Assemblage

The preeminent questions are whether the oil on the sea floor in or near the DWH site was a product of the event, and secondly did that oil cause the changes observed in the polychaete assemblage. Along with the linear relationship between TOE and total PAH, there was also a strong correlation between fluorescence and total PAH reported for water column samples in the plume at the oil spill location (Wade *et al.*, 2011). The PAH composition in the water samples was predominantly petrogenic and thus derived from oil, based on the relative abundance of parent and alkylated PAH. Weathering appeared to have occurred based on the percentage

contributions of total naphthalenes ($TN = \text{naphthalene} + C1 + C2 + C3 + C4$) compared to total phenanthrenes/anthracenes ($TP/A = \text{phenanthrene} + \text{anthracene} + C1 + C2 + C3 + C4$). The TN and total TP/A accounted for 57% and 21% respectively of the total PAH in DWH oil (OSAT 2010). In our sediment material the TN and TP/A accounted for 2 to 5% and 18 to 32% respectively of the total PAH, and this lower percentage of TN likely reflects loss due to the high solubility of naphthalenes in water. This weathering may have occurred prior to deposition because weathered oil had been reported in the water column (Wade *et al.*, 2008). There were also pyrogenic PAH present that may have originated from the fire at the platform, burning of oil at the surface, atmospheric deposition and/or transport from continental sources. The Operational Science Advisory Team demonstrated that PAH concentrations were higher in sediment samples collected in the footprint of the blow out site 4-6 months after the spill (OSAT, 2010), as reported by Montagna *et al.* (2013). However the PAH we detected were from a mixed source.

In the OSAT report, post-oil spill sediment samples that exceeded prior historical benchmarks (in Wade *et al.* 2008) were all located within 0.33-2.7 km of the spill site. Samples however above reference levels were found out to 10 km from the well head. White *et al.* (2012) observed impacts of DWH oil on deep-sea coral 11km southwest of the DWH well head three to four months after the flow was capped, within the linear distance in which we observed a decline in abundance and diversity and a change in dominant species.

As might be expected, the non-selective deposit feeding Capitellidae were relatively pollution-resistant. On the other hand, the percentage of carnivores was correlated positively with an increase in PAH and organic content in *Todosos Santos* Bay, Brazil (Venturini and

Tommasi, 2004), as in our DWH samples, suggesting that carnivorous species are somehow immune to the stress other groups experienced.

Continental slopes are notoriously variable in time and space (Levin and Sibuet, 2011), and the effects of natural oil seeps, seasonality, topography and bottom currents cannot be excluded. Hubbard (1995) for example observed what he thought could be seasonal variation in polychaete abundances at the “C” locations (Fig 1A). Thus our observations could be responses to seasonality in the surface water that is transferred to the sea floor in yet-to-be-observed particle fluxes (Rowe, 2013). Unstable sediments of the shelf and upper slope export organic matter to deep water (Bianchi *et al.*, 2006) and massive slumps can smother mid-slope habitats (Santschi and Rowe, 2008), both of which could also give rise to large differences in species composition. An initial decrease in sensitive species has often been observed in shallow-water oil spills (Sanders *et al.*, 1980), followed by recruitment of opportunistic species, especially capitellids adapted to survive and sometimes to even flourish in organically enriched sediments. Habitats recover but this can take years, based on long-term shallow water observations (Dauvin, 1998; Dutrieux, 1989; Gomez Gesteira and Dauvin, 2000; Gomez Gesteira *et al.*, 2003; Kingston, 2002). The unprecedented depth and amount of oil injected into the deep ocean make predicting the recovery difficult. The lower diversity and abundance five months after the spill is a single point on a temporal continuum, but where this remote habitat is in the continuum remains unknown. A more exhaustive longitudinal study is needed to document succession of dominant macrofaunal species that may have been exposed to deep-water contamination by the DWH event (Whitehead *et al.*, 2011).

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