

Recovery of salt marsh benthic microalgae and meiofauna following the *Deepwater Horizon* oil spill linked to recovery of *Spartina alterniflora*

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ABSTRACT: We examined the effects of the *Deepwater Horizon* oil spill on benthic microalgae and meiofauna in Louisiana, USA, salt marshes. Further, we quantified recovery over 4 yr and compared it to recovery of the dominant salt marsh macrophyte *Spartina alterniflora*. Although impacts were apparent at lightly and moderately oiled sites, negative effects on microalgal biomass and meiofaunal abundance and diversity were strongest in heavily oiled marshes where almost complete mortality of *S. alterniflora* occurred. However, these metrics, as well as photosynthetic pigment composition and meiofauna community composition, indicated substantial recovery ~36 mo post-spill, coincident with recovery of *S. alterniflora* stem density, even in heavily oiled marshes. Meiofaunal diversity (as measured by copepod species richness) in the earliest stages of recovery was highest where recovering *S. alterniflora* stems were most dense, and overall, meiofauna recovery was more closely linked to *S. alterniflora* than to vegetative recovery per se. However, for the polychaete *Manayunkia aestuarina*, ostracods and kinorhynchans, recovery was still ongoing 4 yr after the spill. These findings suggest that the important ecological services provided in support of food webs by benthic microalgae (a principal basal food resource) and meiofauna (a principal consumer of benthic microalgae and dietary resource to higher trophic levels) largely returned coincident with the recovery of *S. alterniflora*. *S. alterniflora* may therefore be a good indicator for the broader recovery of salt marsh infaunal benthos, at least in marshes without accelerated shoreline erosion induced by oiling.

KEY WORDS: *Deepwater Horizon* oil spill · Meiofauna · Benthic microalgae · Salt marsh · *Spartina alterniflora*

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INTRODUCTION

Human activities can severely threaten coastal ecosystems and the many services they provide (Zedler & Kercher 2005, Barbier et al. 2011, Engle 2011). Pollution is often among the most intense anthropogenic stressors and can drive rapid and severe habitat degradation (Pezeshki et al. 2000,

Deegan et al. 2012). Oil spills in particular pose a heightened threat to ecosystem health because they are unpredictable in space and time, and because their impacts are difficult to mitigate. Past oil spills in coastal habitats have led to reduced abundances of plants and animals (Mendelssohn et al. 2012), loss of ecosystem services (Jewett et al. 1999), and persistence of oil-derived compounds that have long-lasting

impacts on communities and food webs (Duke et al. 1997, Peterson et al. 2003).

The blowout of the Macondo MC252 wellhead on 20 April 2010 released a judicially decreed 3.19×10^6 barrels ($0.51 \times 10^6 \text{ m}^3$) of crude oil into the Gulf of Mexico (the *Deepwater Horizon* oil spill). About 1800 km of Gulf Coast shoreline habitats were oiled, with 582 km designated as moderately to heavily oiled (Michel et al. 2013). Approximately 95% of the total marsh oiling occurred in Louisiana, the nation's largest and most productive wetland-estuarine environment. The heaviest oiling was most widespread in *Spartina alterniflora*- and *Juncus roemerianus*-dominated salt marshes located in northern Barataria Bay, Louisiana, USA (Michel et al. 2013). Plant communities experienced strong effects, including heavy mortality that denuded shorelines (Lin & Mendelssohn 2012, Silliman et al. 2012, Khanna et al. 2013, Zengel et al. 2014).

A diverse assemblage of microalgae and meiofauna ubiquitously inhabit sediments in coastal ecosystems. Benthic microalgae produce oxygen and serve as an important basal food resource where light penetrates to the sediment surface (Miller et al. 1996, Grippo et al. 2009). Meiofauna graze on and sometimes regulate microalgae (Carman et al. 1997) while serving as prey for higher trophic levels (McCall & Fleeger 1995, Gregg & Fleeger 1998). Furthermore, benthic microalgae and meiofauna express a range of physiological capabilities, and responses to hydrocarbon toxicity are varied. Some species of meiofauna are very tolerant, while others are very sensitive, especially during reproduction and development (Fleeger & Carman 2011). In a series of microcosm studies conducted with diesel fuel in Louisiana marshes, Carman and co-workers (e.g. Carman et al. 1997, 2000b, Millward et al. 2004) found that benthic microalgae are more tolerant than most meiofauna, and that responses are species-specific. Indirect effects, such as toxicant-induced changes in community grazing rate, nutrient flux and interspecific competition, were invoked as causes of changes in population size for benthic microalgae and tolerant meiofauna (Carman et al. 2000a, Fleeger et al. 2003). Benthic microalgae and meiofauna are therefore significant indicators of ecosystem function and of effects and recovery from oil spills.

Previous studies investigating effects of oil spills on salt marshes indicate that negative impacts on plants may be mitigated by vegetation regrowth once oil has degraded (Mendelssohn et al. 2012, Silliman et al. 2012). This finding suggests that marshes are intrinsically resilient (i.e. able to recover from oiling)

(Michel & Rutherford 2014), dependent on the degree and nature of oiling conditions and in the absence of accelerated oil-induced shoreline erosion (Silliman et al. 2012, McClenahan et al. 2013). Benthic microalgal responses to crude and fuel oil spills vary, ranging from decreases in biomass and primary production (Lee & Lin 2013) to dramatic increases in biomass (Carman et al. 1997). Meiofauna may persist after or quickly recover from spills (Giere 2009), although variation in factors such as sediment type, oiling intensity, chemical composition, and toxicity affect the time to recovery. For example, recovery following the Amoco Cadiz oil spill along the coast of France took ~6 yr (Boucher 1985, Bodin 1988). Studies of restored salt marshes indicate that the recovery rates of plants and benthic invertebrates may differ (Warren et al. 2002, Able et al. 2008, Staszak & Armitage 2013). It is therefore important to establish rates of microalgal and infaunal recovery to determine if the ecological functions provided by small benthic producers and consumers return with recovery of the plant community (Pennings et al. 2014).

The purpose of this study was to quantify the effects of the *Deepwater Horizon* oil spill on benthic microalgae and meiofauna. We also monitored recovery over a period of 4 yr. Lin & Mendelssohn (2012, unpubl. data) and Zengel et al. (2015) studied the response of the plant community including the dominant salt marsh macrophyte *S. alterniflora* (hereafter *Spartina*) in the same sampling stations as used here, and we compared the responses of *Spartina* with those of benthic microalgae and meiofauna to determine if recovery was interrelated.

MATERIALS AND METHODS

Field sites

Our field studies were carried out in coastal salt marshes in northern Barataria Bay, Louisiana. This area became one of the most severely oiled along the Gulf of Mexico coast when Macondo oil entered Barataria Bay about 2 mo after the spill began. Two sets of sampling stations were established (but with different objectives and therefore with different sample designs) within 3 m of the shoreline in the microtidal intertidal zone. 'Shoreline cleanup assessment technique' data, field observations, and subsequently, our measures of total petroleum hydrocarbons (TPH) were used to assign an oiling intensity level at each station.

'Short-term sites' were located between coordinates 29.44064–29.4449° N and 89.89014–89.89000° W, and consisted of replicated ($n = 4$), 50 m² plots located along a linear shoreline distance of ~800 m. These plots were established to test oiled marsh cleanup treatments during the emergency response; the site and treatments are described in Zengel et al. (2014, 2015). Short-term sites were sampled collaboratively with other researchers, and the microalgal and meiofauna samples taken were the earliest available from heavily oiled salt marshes in the region. Data from 2 treatments are presented here; one designated 'lightly oiled' and the other 'heavily oiled'. Lightly oiled plots (called 'reference plots' in Zengel et al. 2014, 2015) were randomly located along the nearest contiguous and comparable section of shoreline with lighter to no oiling and intact salt marsh vegetation, located adjacent to the heavily oiled treatment test area with near total marsh vegetation mortality. Heavily oiled plots (called 'no treatment set-asides' and 'oiled controls' in Zengel et al. 2014, 2015) were randomly located within the continuous heavy oiling band comprising the treatment test area. It would have been desirable to have the lightly oiled plots randomly interspersed among the heavily oiled plots, but this was impossible due to the distribution of heavy oiling. Given that reference and lightly oiled plots were spatially clustered, it is possible that factors other than oiling contributed to differences between plots. However, we believe that the large differences in TPH are the most plausible explanation for the large differences in the benthic community we observed (see 'Results'). The plant community at these sites was surveyed 17 and 29 mo after the spill (Zengel et al. 2015), and benthic samples were taken 13, 22 and 29 mo after the spill.

'Long-term sites' consisted of 21 individual stations located over a much larger area (8 × 5 km, between co-ordinates 29.44060–29.47459° N and 89.88492–89.94647° W). Sampling sites were selected with the explicit objective of determining effects and recovery of biota exposed to different oiling intensities, and were randomly stratified among 3 partially interspersed oiling categories within the designated sampling area; 7 received no visible oiling, designated 'reference', 7 were 'moderately oiled' and 7 were 'heavily oiled'. Because oil was primarily transported into the estuary by south and southeastern winds, heavily oiled sites generally occurred on south and southeast facing shorelines, while moderately oiled sites generally occurred on adjacent tangential shorelines. Reference sites were located

along north- and south-facing shorelines located ca. 0.5 to 4 km from oiled stations. The benthic community at long-term sites was sampled on 7 occasions from 18 to 48 mo after the spill. In addition, samples of the plant community were taken on these same dates as well 9 mo after the spill (Lin & Mendelssohn 2012, unpubl data). No oil cleanup treatments were applied in the plots or sampling stations used here.

TPH analysis

Surface soils (0 to 2 cm) were collected from each station on each collection date, transported to our laboratory on ice, extracted with dichloromethane, and analyzed gravimetrically. Extracts were transferred to pre-weighed dishes, dichloromethane was evaporated, and the unevaporated oil remaining in the dishes was weighed to the nearest 0.0001 g. TPH concentration was calculated and expressed as mg g⁻¹ dry soil.

Vegetation

At the short-term sites, % cover of *Spartina* was estimated visually across each experimental plot (data on the full plant community are available in Zengel et al. 2015). At the long-term sites, vegetation samples were taken on each sampling date within a haphazardly located 0.25 m² quadrat following Lin & Mendelssohn (2012). Plants rooted within the quadrat were clipped to the ground surface and separated into live and dead components by species. *Spartina* stem density was determined by counting the number of intact living stems. The above-ground portion of each species, including *Spartina*, was then dried to a constant mass at 60°C and weighed.

Benthic microalgae

A hand-held corer (inner diameter = 1.2 cm) was used to take 2 sediment cores to a depth of 3 mm at haphazardly selected locations at each plot or station to sample the benthic microalgae. The 2 cores were combined in a single sample container and immediately frozen on dry ice. In the laboratory, samples were stored at -80°C.

Photosynthetic pigments were extracted from entire sediment samples with 4 ml of 100% acetone. The sediment-acetone mixture was sonicated for 30 s. The mixture was refrigerated overnight before

centrifugation for 10 min. Samples were filtered (0.2 μm) before analysis. High pressure liquid chromatography (HPLC) was used to examine photosynthetic pigment concentration and composition (Buffan-Dubau & Carman 2000). A Hewlett Packard 1100 liquid chromatograph consisting of a 100 ml loop autosampler, a quaternary solvent delivery system coupled to a diode array spectrophotometer, and a Hewlett Packard 1046A fluorescent detector were used. Hewlett Packard HPChem-Station software was used for data analysis. Chlorophyll *a* (chl *a*), fucoxanthin, zeaxanthin and pheophytin were examined in detail. Pigment concentration (in $\mu\text{g sample}^{-1}$) was converted to $\mu\text{g cm}^{-2}$ based on corer diameter.

Meiofauna

A hand-held corer (inner diameter = 3.5 cm) was used to take 2 sediment cores to a depth of 2 cm at haphazardly selected locations at each plot or station to sample the meiofauna. Both cores were combined into a single sample cup and fixed in 4% formalin. Cups were shaken to break up soil clumps and to mix with formalin. Formalin was replaced after ~24 h, and a solution of Rose Bengal was added. Just prior to sorting, samples were rinsed through a 500 μm sieve stacked on top of a 32 μm sieve. Fauna retained on the 32 μm sieve were extracted from sediments using Ludox; 80 ml of Ludox was added to the sample, which was thoroughly shaken to mix sediment and Ludox. Samples were allowed to settle for 60 to 90 min before the supernatant was carefully poured through a 32 μm sieve and rinsed. This procedure was repeated 2 additional times.

A stereo-dissecting microscope was used to identify and enumerate meiofauna to higher taxonomic rank (e.g. nematodes, copepods, polychaetes) while simultaneously enumerating the sabellid polychaete *Manayunkia aestuarina*. Adult and juvenile copepods were picked from the sample, and adults were later identified to species. Density was standardized to the number of individuals 10 cm^{-2} based on corer diameter.

A total of 24 samples of benthic microalgae and meiofauna were examined from the short-term sites (2 oiling intensities \times 4 replicates \times 3 sampling dates). At the long-term sites, a total of 147 samples of microalgae were initially processed (3 oiling intensities \times 7 replicates \times 7 sampling dates). However, some microalgal samples from moderately and heavily oiled sites were deemed to have an oil content sufficient to harm the HPLC column and were

not analyzed. Thus, sample size varied and ranged from 2 to 7 per oiling intensity category. For the meiofauna, 4 of the 7 samples collected in the field at each oiling category were randomly selected for processing, although 2 additional replicates from each oiling category were processed from the 30 mo collection when an anomalous copepod species composition was observed. A total of 90 meiofauna samples were examined.

Statistical analysis

Univariate techniques with SPSS software (version 22) were employed to analyze plant data, microalgal biomass, meiofauna density, and the number of copepod species. Repeated measures, 2-way ANOVA (with intensity and time as the main effects and an oiling \times time interaction) and Mauchly's test of sphericity were conducted on data from the short-term sites. Sphericity failed (i.e. Mauchly's test statistic was <0.05) in 2 tests (for copepod species richness and polychaete, excluding *M. aestuarina*, density), and the Greenhouse-Geisser correction was used to adjust degrees of freedom. Two-way ANOVA (with oiling and time as the main effects and with a oiling \times time interaction) was conducted on faunal and plant data from the long-term sites and *Spartina* % cover from the short-term sites. Abundance data were $\log_{10}(x + 1)$ transformed before analysis. Analysis of the microalgal biomass at the short-term sites was precluded because of missing data (samples from some plots were not analyzed because high oil content precluded HPLC analysis). Meiofaunal samples from only 4 of 7 long-term sites were routinely processed while *Spartina* counts were available from all 7 sites, and here we analyzed *Spartina* stem density only from sites where meiofauna samples were also analyzed. Tukey's HSD test was used in a *posteriori* comparisons, and results were considered significant at $p < 0.05$ in all statistical tests. The partial Eta-squared method to determine effects sizes was employed for all tests.

Algal photosynthetic pigment composition was examined by analysis of similarity (ANOSIM) using PRIMER software (Clarke & Gorley 2006). A 2-way crossed design with replication, testing for effects of oiling and time, was employed at both the short-term and long-term sites. Pigments that contributed to differences among stations or sampling dates (% contribution to dissimilarity) were determined using 2-way similarity percentages (SIMPER) analysis, also using PRIMER software. Chl *a*, fucoxanthin, pheo-

phytin and zeaxanthin concentrations were square-root transformed and the Bray-Curtis resemblance index was employed. ANOSIM and SIMPER procedures correspondingly were also applied in separate analyses of the meiofauna community as represented by major taxon abundance (the 16 most abundant taxa; see 'Results') at the short-term and long-term sites. In addition, multi-dimensional scaling (MDS) ordination was used to visualize differences among sites and over time in the meiofaunal community. Two dimensions for ordinations were configured after 50 iterations. Selected sampling dates from both the short-term and long-term sites were compared in ordination plots (see the Supplement at www.int-res.com/articles/suppl/m536p039_supp.pdf). All error terms throughout the text are expressed as standard error (SE). Raw data for benthic microalgae and meiofauna can be found at the following links: doi: 10.7266/N7P26W1S, doi:10.7266/N7DN430F, doi:10.7266/N7J9649F.

RESULTS

TPH was well differentiated among reference and oiled sites (Table 1). Mean TPH at the short-term sites through 29 mo was 6.2 ± 1.5 in lightly oiled, and 406.1 ± 60.6 mg TPH g^{-1} in heavily oiled plots. Mean TPH at the long-term sites through 48 mo was 0.35 ± 0.04 at reference, 17.0 ± 5.69 in moderately, and 171.9 ± 35.9 mg g^{-1} in heavily oiled marshes. TPH in heavily oiled sites declined from ~ 500 at 9 mo to ~ 100 mg g^{-1} by 48 mo.

Table 1. Mean (\pm SE) total petroleum hydrocarbons (mg TPH g^{-1} sediment) at reference, lightly, moderately, and heavily oiled study sites in northern Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. (–) not applicable

Months after spill	Reference	Lightly oiled	Moderately oiled	Heavily oiled
Short-term sites (n = 4)				
13	–	6.1 ± 3.8	–	449.4 ± 90.6
22	–	3.7 ± 0.9	–	344.2 ± 95.0
29	–	8.9 ± 2.1	–	424.7 ± 145.0
Long-term sites (n = 7)				
9	0.58 ± 0.12	–	70.0 ± 38.4	510.7 ± 231.1
18	0.3 ± 0.10	–	16.9 ± 8.7	208.0 ± 51.8
24	0.7 ± 0.19	–	15.2 ± 9.0	161.8 ± 45.7
30	0.2 ± 0.05	–	4.0 ± 1.8	62.0 ± 28.5
36	0.3 ± 0.08	–	20.8 ± 11.6	151.8 ± 59.0
40	0.3 ± 0.04	–	2.6 ± 1.1	51.0 ± 43.5
42	0.2 ± 0.05	–	3.4 ± 1.1	130.0 ± 50.7
48	0.32 ± 0.06	–	3.2 ± 1.6	99.9 ± 52.2

Spartina was the dominant species of vegetation in lightly oiled plots at the short-term sites, comprising $>90\%$ of vegetation cover (Zengel et al. 2015). *Juncus roemerianus* and *Spartina* were co-dominant at the long-term sites at reference sites (Lin & Mendelssohn 2012) and together comprised $\sim 89\%$ of the live biomass. Across all samples, photosynthetic pigments ranked from highest to lowest concentration were chl *a* (which serves as a proxy for microalgal biomass), fucoxanthin, pheophytin and zeaxanthin. Nematodes were the most abundant taxon, comprising $\sim 85\%$ of the total meiofauna across all samples. Polychaetes were dominated by adults and juveniles of *Manayunkia aestuarina* (5% of the total), and juveniles mostly belonging to the family Spionidae (3%). Adult and juvenile copepods averaged about 5% of the total, and copepod nauplii were frequently observed. Although found at all sites, kinorhynchs (0.5%) were more abundant at the long-term sites, while ostracods (0.3%) were more abundant at the short-term sites. All kinorhynchs in a subset of ~ 100 individuals were identified as *Echinoderes coulli*, a species previously reported in US salt marshes (Higgins & Fleeger 1980). Other less common taxa included mites, hydrozoans, oligochaetes, and gastrotrichs. Juveniles of isopods, tanaids, amphipods, bivalves, and gastropods also occurred, but in low abundances. All 16 taxa mentioned here were included in ANOSIM calculations as members of the meiofaunal community. Most of the copepod species we observed have been reported in similar habitats in the northern Gulf of Mexico (Suárez-Morales et al. 2009). A total of 23 copepod species were identified. The most abundant and commonly observed species were *Nitokra lacustris*, *Schizopera knabeni*, *Enhydrosoma* sp., *Mesochra mexicana*, *Leptocaris brevicornis*, *Nannopus palustris*, and *Onychocamptus mohammed*.

Short-term sites

Spartina % cover in lightly oiled plots was 89.6 ± 2.54 at 17 mo, and $87.2 \pm 3.50\%$ at 29 mo after the spill. *Spartina* % cover in heavily oiled plots was $0.3 \pm 0.14\%$ at 17 mo and increased to only $1.2 \pm 0.81\%$ at 29 mo, although vegetation covered $\sim 20\%$ of the plots (Zengel et al. 2015). *Spartina* % cover differed between the lightly and heavily oiled plots but did not differ over time (2-way ANOVA, $p = 0.000$; Table 2). The effects size for oiling intensity was 0.995.

Table 2. Results of ANOVA testing for oiling intensity and time effects for benthic microalgal biomass, meiofauna abundance and copepod species richness at reference, lightly, moderately, and heavily oiled study sites in northern Barataria Bay, Louisiana, following the *Deepwater Horizon* oil spill (see 'Materials and methods' for details). p-values ($p < 0.05$ in **bold**) are reported for all tests as main effects of oiling intensity and time, and as an oiling \times time (O \times T) interaction. Effects sizes were determined as partial Eta-squared. *Spartina* % cover and ostracods were tested at the short-term sites, and *Spartina* stem density and kinorhynchs at the long-term sites. (–) not applicable

Level	Short-term sites		Long-term sites	
	p-value	Effects size	p-value	Effects size
<i>Spartina</i> % cover/stem density				
Oil	0.000	0.995	0.011	0.121
Time	1.0	0.0	0.025	0.184
O \times T	1.0	0.0	0.736	0.110
Microalgal biomass				
Oil	–	–	0.893	0.002
Time	–	–	0.008	0.149
O \times T	–	–	0.064	0.166
Total meiofauna				
Oil	0.003	0.798	0.029	0.097
Time	0.315	0.177	0.001	0.277
O \times T	0.857	0.026	0.081	0.230
Nematodes				
Oil	0.004	0.776	0.089	0.068
Time	0.889	0.019	0.011	0.209
O \times T	0.888	0.020	0.089	0.227
Copepods				
Oil	0.007	0.731	0.533	0.018
Time	0.013	0.514	0.000	0.323
O \times T	0.666	0.066	0.131	0.211
Copepod species richness				
Oil	0.009	0.711	0.483	0.021
Time	0.011	0.656	0.000	0.301
O \times T	0.365	0.143	0.028	0.268
Polychaetes (except <i>M. aestuarina</i>)				
Oil	0.001	0.867	0.523	0.019
Time	0.007	0.720	0.000	0.596
O \times T	0.125	0.343	0.027	0.269
<i>Manayunkia aestuarina</i>				
Oil	0.000	0.889	0.000	0.375
Time	0.003	0.619	0.065	0.154
O \times T	0.009	0.546	0.908	0.080
Ostracods/kinorhynchs				
Oil	0.000	0.896	0.000	0.501
Time	0.050	0.393	0.037	0.172
O \times T	0.019	0.486	0.113	0.217

Benthic samples were first collected at the short-term sites 13 mo after the spill began (11 mo after marsh oiling). Benthic microalgal biomass in the heavily oiled plots at 13 mo was very low (near zero) and was the lowest (by a factor ≥ 15) observed among all of our collections at the short- and long-term sites (Fig. 1a). Although microalgal biomass in the heavily oiled plots increased by ~ 40 times from 13 to 22 mo, biomass remained $\sim 30\%$ lower in the heavily oiled

compared to lightly oiled plots. However, microalgal biomass was similar in the heavily oiled and lightly oiled plots at 29 mo (Fig. 1a).

Algal photosynthetic pigment composition differed between lightly and heavily oiled plots (ANOSIM, $p = 0.004$), and pigment composition varied over time (ANOSIM, $p = 0.025$). Pigment composition at 13 and 29 mo differed ($p = 0.0021$), as did composition at 22 and 29 mo ($p = 0.0026$). SIMPER indicated that chl *a* contributed 40.9% of the dissimilarity between lightly and heavily oiled plots.

Among all of our samples at the short- and long-term sites, lowest densities of the sum total of meiofauna (hereafter total meiofauna), the 3 most abundant individual taxa (i.e. nematodes, copepods, polychaetes), and several rare taxa, e.g. ostracods, were all observed in heavily oiled plots 13 mo after the spill (Figs. 2a–8a). For example, total meiofauna averaged 761.6 ± 122.93 in lightly but 182.0 ± 49.03 ind. 10 cm^{-2} in heavily oiled plots (a 76% reduction). Copepod species richness in heavily oiled plots at 13 mo was also the lowest observed, and was lower by $\sim 50\%$ than in lightly oiled plots (Fig. 5a). Polychaetes, amphipods and tanaids were absent from all plots 13 mo after the spill (Figs. 6a & 7a), but polychaetes were present in all later samples, and amphipods and tanaids frequently occurred in later collections.

Density trends among the meiofauna differed over time and between oiling categories. Total meiofauna and nematode densities varied little between 13 and 29 mo after the spill in both oiling categories, while the density of copepods in heavily oiled plots increased 6 \times over the same period. Polychaete density, excluding *M. aestuarina*, was highest 22 mo after the spill in both oiling categories. However, densities of *M.*

aestuarina and ostracods in heavily oiled plots remained close to zero throughout the 29 mo period (Figs. 6a & 8a).

Total meiofauna, nematodes, copepods, polychaetes, and ostracods were all significantly lower in density in heavily than in lightly oiled plots (repeated-measures ANOVA; Table 2, Figs. 2a–8a). Copepod species richness was also significantly lower in heavily oiled plots (Table 2, Fig. 5a). Poly-

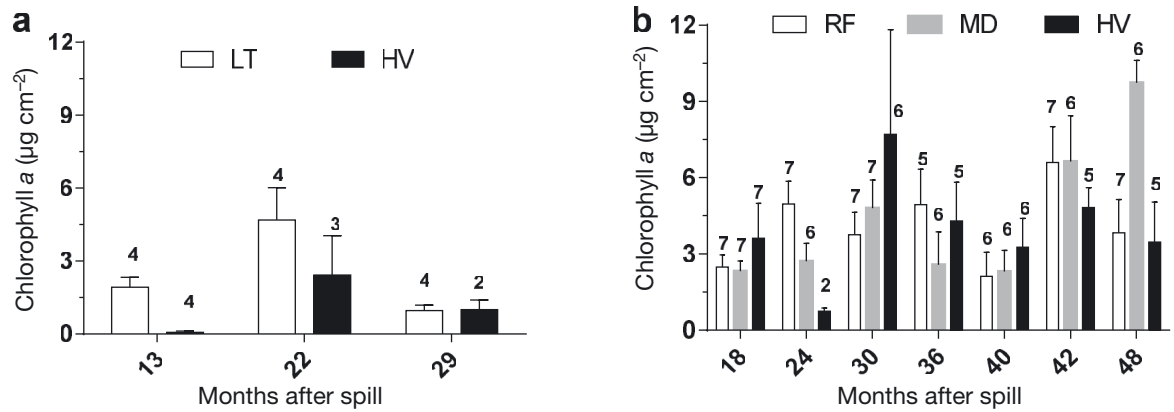


Fig. 1. Mean (\pm SE) chlorophyll *a* concentration ($\mu\text{g chl } a \text{ cm}^{-2}$) at reference (RF), lightly (LT), moderately (MD), and heavily (HV) oiled study sites in northern Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. (a) Short-term sites; (b) long-term sites. Sample size is displayed over the bars

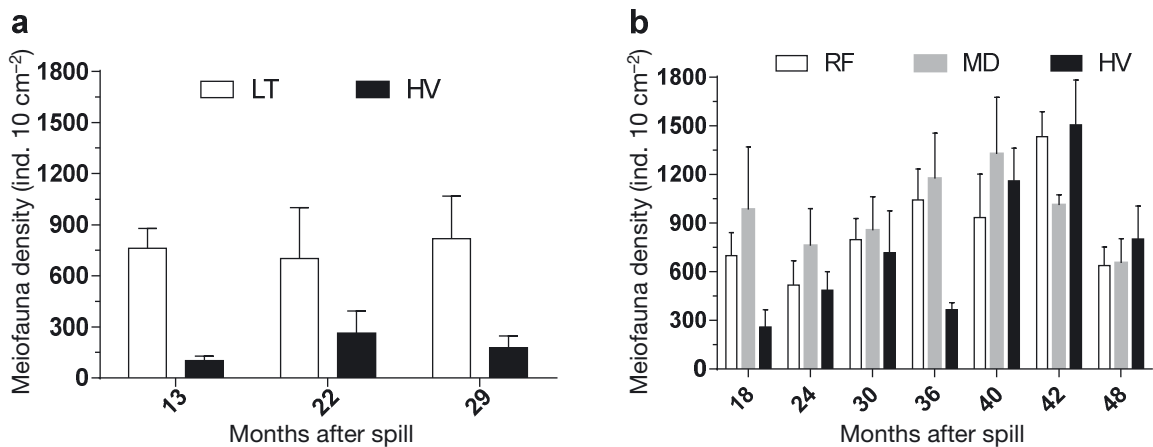


Fig. 2. Mean (\pm SE, $n = 4$ except for the 30 mo collection when $n = 6$) density of total meiofauna (ind. 10 cm^{-2}) at reference (RF), lightly (LT), moderately (MD), and heavily (HV) oiled study sites in northern Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. (a) Short-term sites; (b) long-term sites

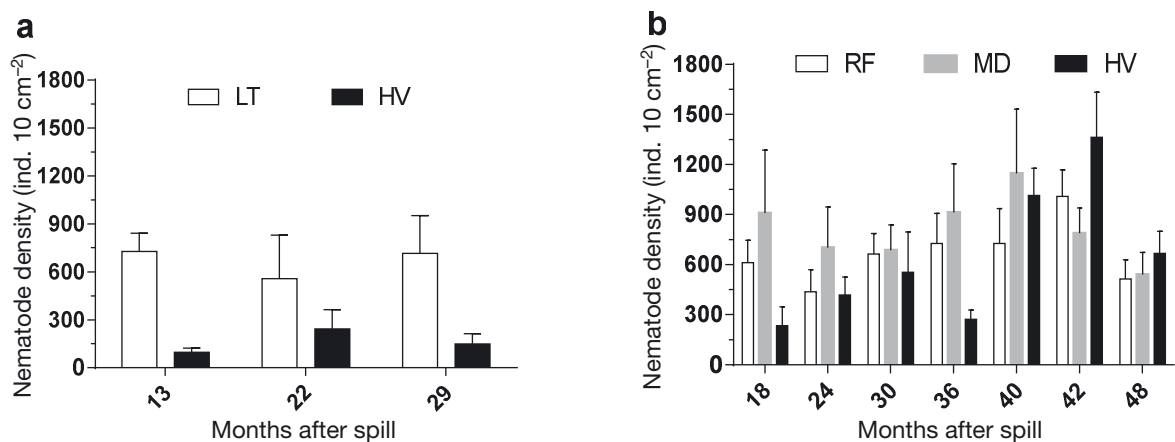


Fig. 3. Mean (\pm SE, $n = 4$ except for the 30 mo collection when $n = 6$) density of nematodes (ind. 10 cm^{-2}) at reference (RF), lightly (LT), moderately (MD), and heavily (HV) oiled study sites in northern Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. (a) Short-term sites; (b) long-term sites

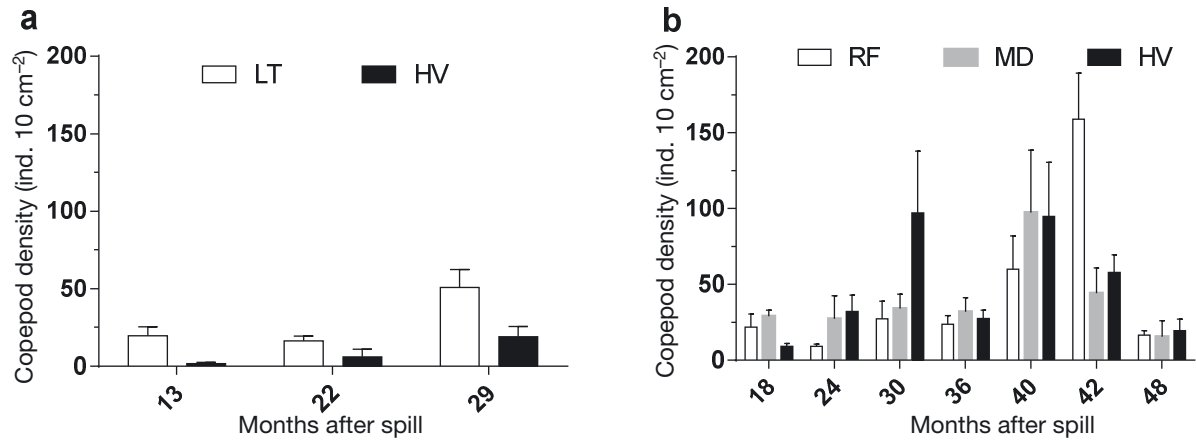


Fig. 4. Mean (\pm SE, $n = 4$ except for the 30 mo collection when $n = 6$) density of copepods (ind. 10 cm⁻²) at reference (RF), lightly (LT), moderately (MD), and heavily (HV) oiled study sites in northern Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. (a) Short-term sites; (b) long-term sites

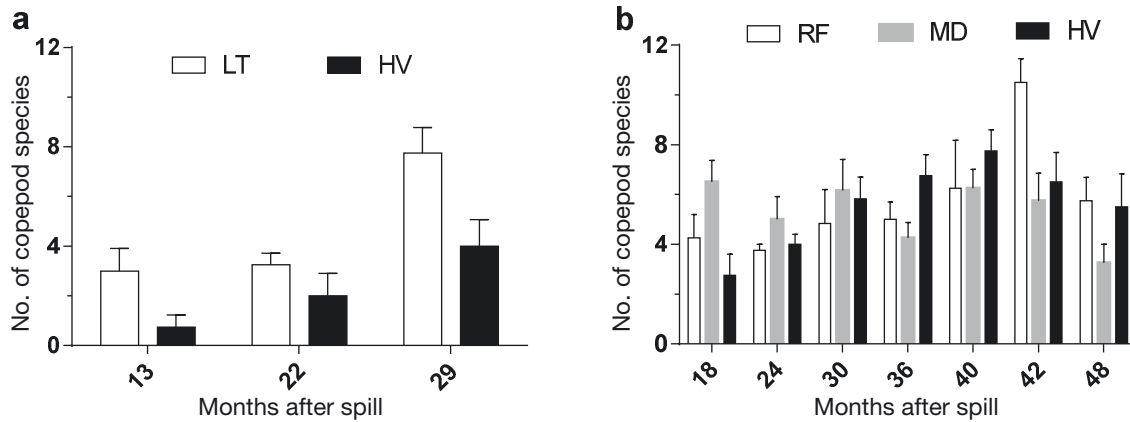


Fig. 5. Mean (\pm SE, $n = 4$ except for the 30 mo collection when $n = 6$) number of copepod species (no. sample⁻¹) at reference (RF), lightly (LT), moderately (MD), and heavily (HV) oiled study sites in northern Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. (a) Short-term sites; (b) long-term sites

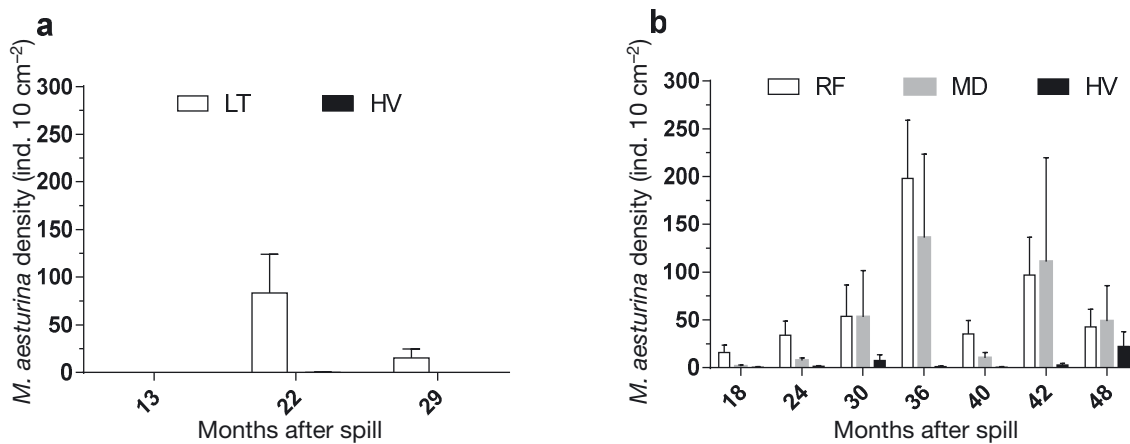


Fig. 6. Mean (\pm SE, $n = 4$ except for the 30 mo collection when $n = 6$) density of *Manayunkia aestuarina* (ind. 10 cm⁻²) at reference (RF), lightly (LT), moderately (MD), and heavily (HV) oiled study sites in northern Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. (a) Short-term sites; (b) long-term sites

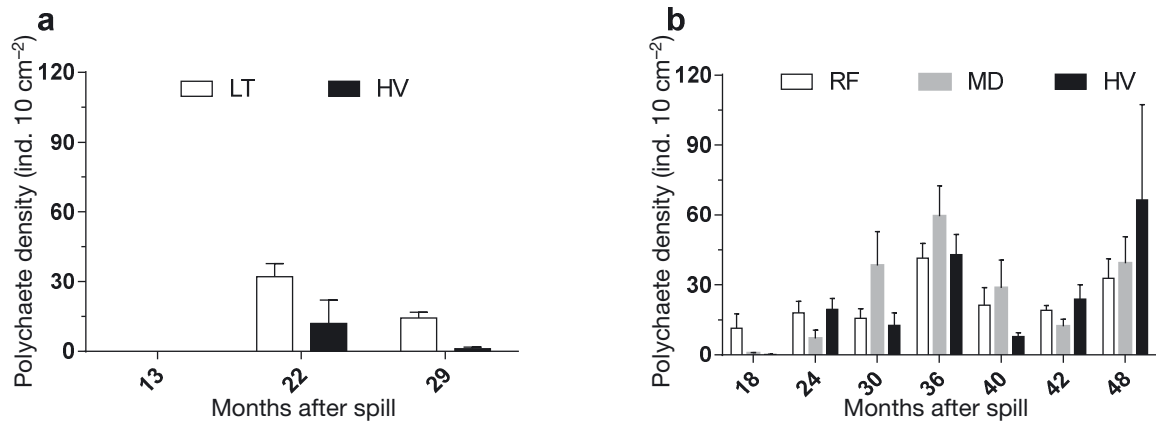


Fig. 7. Mean (\pm SE, $n = 4$ except for the 30 mo collection when $n = 6$) density of polychaetes excluding *M. aestuarina* (ind. 10 cm^{-2}), at reference (RF), lightly (LT), moderately (MD), and heavily (HV) oiled study sites in northern Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. (a) Short-term sites; (b) long-term sites

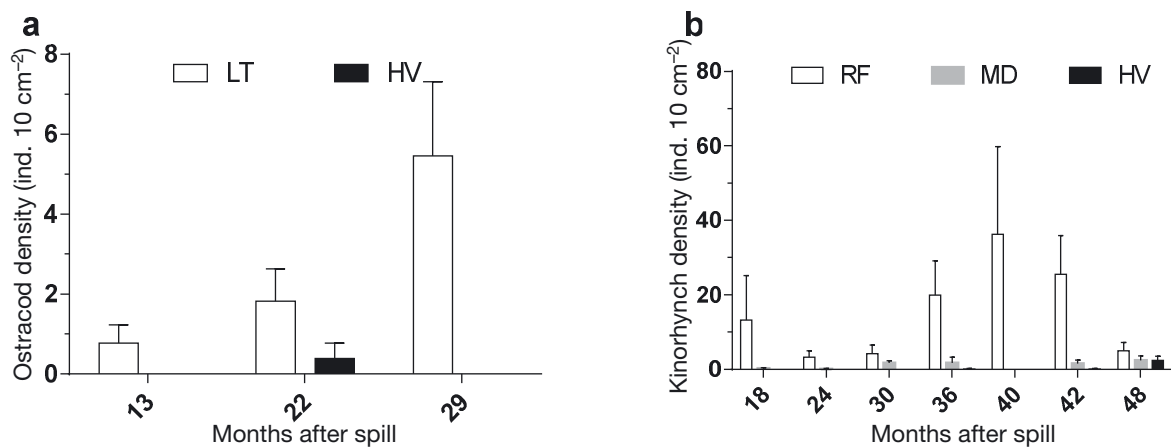


Fig. 8. (a) Mean (\pm SE, $n = 4$ except for the 30 mo collection when $n = 6$) density of ostracods (ind. 10 cm^{-2}) at reference (RF), lightly (LT), moderately (MD), and heavily (HV) oiled study sites in northern Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. (a) Short-term sites; (b) long-term sites

chaetes, copepods, and ostracods significantly increased in density over time (Table 2, Figs. 2a–8a), as did copepod species richness. *M. aestuarina* and ostracod densities were significantly higher in lightly than heavily oiled plots in collections at 22 and 29 mo, but not at 13 mo (the oiling \times time interaction was significant; Table 2). Effects sizes associated with oiling for all repeated measures tests were >0.71 , and effects sizes for time, when significant, were >0.50 .

The meiofaunal community, as measured by major taxon composition, differed between lightly and heavily oiled plots (ANOSIM, $p = 0.001$) and over time (ANOSIM, $p = 0.001$). Community similarity differed between 13 and 22 mo ($p = 0.002$) and between 13 and 29 mo ($p = 0.001$). Copepods, nematodes and *M. aestuarina* contributed most to the dissimilarity between lightly and heavily oiled plots, while poly-

chaetes (excluding *M. aestuarina*), copepods, and *M. aestuarina* contributed most to dissimilarities over time (SIMPER analysis). MDS of samples from 13 and 22 mo after the spill clearly separated the meiofaunal community between lightly and heavily oiled plots and over time (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m536p039_supp.pdf). Heavily oiled samples at 13 and 22 mo were similar to each other and were primarily aggregated in the center of the ordination space.

Long-term sites

Although the first benthic sampling at the long-term sites was taken 18 mo after the spill (16 mo after marsh oiling), the plant community was sampled at 9 mo after the spill (Lin & Mendelssohn 2012).

Spartina stem density was very low in heavily oiled samples taken 9 mo after the spill, averaging 0.57 ± 0.57 stems m^{-2} . Stem densities increased to 140 ± 59.5 stems m^{-2} by 18 mo in the same heavily oiled sites that were sampled for meiofauna (Fig. 9). Stem densities at reference and heavily oiled sites fluctuated over time (with lows in spring and highs in fall), but means were similar in samples taken from 18 mo and thereafter. However, stem densities in moderately oiled marshes were higher (by 1.5 to 2 times) than reference or heavily oiled sites from 18 to 30 mo after the spill. After 36 mo, stem densities became more similar in all oiling categories.

Oiling and time both affected *Spartina* stem density (2-way ANOVA; Table 2). Stem densities in moderately oiled marshes were significantly greater than in heavily oiled marshes (Tukey's HSD test, $p = 0.041$). Although the effect of time was significant, Tukey's test did not indicate a significant difference between any paired comparisons among sampling dates. Effects sizes for main effects and the oiling \times time interaction were <0.25 . Lin & Mendelssohn (unpubl. data) examined an extended vegetation dataset from the long-term sites that included samples taken 9 mo after the spill and a sample size of 7 and concluded that, based on stem density, *Spartina* recovered ~ 36 mo after the spill.

Benthic microalgal biomass tended to be low in collections in moderately and heavily oiled marshes at 18 or 24 mo after the spill but then generally increased (Fig. 1b). Peak biomass was higher by $\sim 4\times$

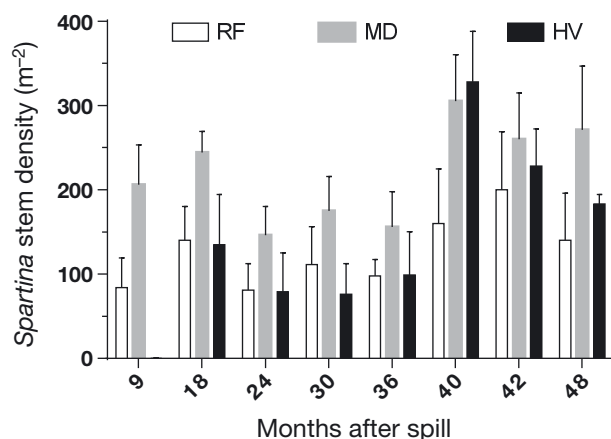


Fig. 9. Mean (\pm SE, $n = 4$ except for the 9 mo collection when $n = 7$ and the 30 mo collection when $n = 6$) of *Spartina alterniflora* stem density (no. m^{-2}) at the long-term sites for reference (RF), moderately (MD), and heavily (HV) oiled study sites in northern Barataria Bay, Louisiana, USA, following the *Deepwater Horizon* oil spill. Except for the 9 mo collections, samples used in this figure were from the same sites as samples of benthic microalgae and meiofauna

compared to the lowest values at 18 or 24 mo. Microalgal biomass in reference marshes varied over time; the highest value was 3 to 4 times greater than the lowest. Microalgal biomass was not affected by oiling although significant variation over time was detected (2-way ANOVA, $p = 0.008$; Table 2). Across time, microalgal biomass differed only between collections at 40 and 42 mo after the spill (Tukey's test). Effects sizes were <0.15 in this test.

However, oiling affected algal photosynthetic pigment composition (ANOSIM, $p = 0.013$); heavily oiled sites differed from reference ($p = 0.039$) and moderately oiled ($p = 0.008$) sites. Photosynthetic pigment composition also differed among sampling times (ANOSIM, $p = 0.001$). Pigment composition at 18 mo differed from all other sampling times except 24 mo; pigments at 24 mo also differed from 42 and 48 mo, and pigments at 30 mo also differed from 48 mo (ANOSIM post-hoc tests; Table 3). Chl *a* contributed most to dissimilarities between oiling and time, always $>50\%$ of the total (SIMPER).

The lowest densities at the long-term sites for total meiofauna, nematodes, copepods (and the number of copepod species), polychaetes, and kinorhynchs were all observed in heavily oiled marshes 18 mo

Table 3. Results of ANOSIM post hoc tests examining differences among collections taken over time (in months) after the *Deepwater Horizon* oil spill at the long-term sites. p -values ($p < 0.05$ in **bold**) of tests conducted on algal photosynthetic pigment composition and the composition of major taxa of meiofauna are presented

Sample comparison	Pigment composition	Meiofauna composition
18, 24	0.084	0.025
18, 30	0.008	0.001
18, 36	0.038	0.002
18, 40	0.007	0.003
18, 42	0.001	0.001
18, 48	0.001	0.002
24, 30	0.441	0.42
24, 36	0.773	0.007
24, 40	0.174	0.01
24, 42	0.032	0.001
24, 48	0.001	0.059
30, 36	0.16	0.255
30, 40	0.122	0.656
30, 42	0.554	0.258
30, 48	0.009	0.184
36, 40	0.284	0.002
36, 42	0.071	0.001
36, 48	0.012	0.163
40, 42	0.002	0.024
40, 48	0.004	0.002
42, 48	0.001	0.004

after the spill (Figs. 2b–8b). However, total meiofaunal densities across all sampling dates were highest in moderately oiled marshes; overall means were 861.9 ± 75.94 in reference, 959.5 ± 94.32 in moderately, and 753.5 ± 102.09 ind. 10 cm^{-2} in heavily oiled marshes (Fig. 2b).

Density trends after 18 mo differed among meiofaunal taxa and among oiling categories. For total meiofauna and nematodes from 18 to 30 mo after the spill, the highest densities were consistently observed in moderately oiled marshes (where densities averaged ~ 1.5 times higher than at reference sites), and lowest densities were found in heavily oiled marshes (where densities were $\sim 24\%$ lower than at reference sites). Densities increased by 30 to 75% thereafter, but declined by $\sim 40\%$ at 48 mo (Figs. 2b & 3b). Copepod and polychaete densities were highly variable over time. Copepod densities remained low from 18 to 36 mo in all oiling categories except for a singular ~ 4 times increase at 30 mo in heavily oiled marshes. This increase in heavily oiled sites at 30 mo was caused by a pronounced increase in density of 1 species, *Harpacticus* sp., which increased from <1 to 42.9% of the copepod fauna. Copepod species composition and relative abundances were otherwise very similar throughout all sampling sites and times. Copepod densities increased by ~ 4 times after 36 mo in all oiling categories, but declined by 25 to 75% at 48 mo (Fig. 4b). Polychaete densities, excluding *M. aestuarina*, remained low until pronounced increases (by 3 to 4 times) occurred by 30 to 36 mo; densities between 36 and 42 mo declined by 30 to 50% from this peak but increased by 30 to 70% by 48 mo (Fig. 7b). *M. aestuarina* densities steadily increased from 18 to 36 mo in reference and moderately oiled marshes, but fluctuated (by a factor of 2 to 3) from collection to collection thereafter (Fig. 6b). However, *M. aestuarina* density in heavily oiled marshes remained very low over time (for example at 42 mo, mean density of *M. aestuarina* was 97.0 ± 39.7 in reference and 3.0 ± 1.5 ind. 10 cm^{-2} in heavily oiled marshes) until a modest increase (to 22.5 ± 15.5 ind. 10 cm^{-2} , a value $\sim 50\%$ lower than at reference sites) was observed at 48 mo. Kinorhynch densities were also highly variable within and among collections over time in reference sites, varying by as much as a factor of 5 (Fig. 8b). Kinorhynch densities in moderately and heavily oiled marshes were, however, much lower ($\geq 90\%$) than in reference marshes through 42 mo (for example, mean density of kinorhynchs was 25.5 ± 10.4 in reference and 0.13 ± 0.13 ind. 10 cm^{-2} in heavily oiled marshes at 42 mo) but modestly increased (to 2.3 ± 1.1 ind. 10 cm^{-2} at

the heavily oiled sites, a value $\sim 50\%$ lower than at reference sites) at 48 mo. Copepod species richness generally increased over time at reference and heavily oiled marshes (the difference from the lowest to the highest values was 2 to 3 times; Fig. 5b). However, the highest copepod species richness in the earliest collections after the spill, 18 to 30 mo, was observed at moderately oiled sites where values were about ~ 1.5 times higher than at reference or heavily oiled sites.

Oiling significantly affected the densities of total meiofauna, *M. aestuarina*, and kinorhynchs (2-way ANOVA; Table 2). For total meiofauna, density was significantly greater at moderately compared to heavily oiled sites (Tukey's test). *M. aestuarina* density differed among all oiling categories. For kinorhynchs, density at reference sites was greater than at both moderately and heavily oiled sites. The density of polychaetes (excluding *M. aestuarina*) and copepod species richness may have responded to oiling differently at different sampling times (the oiling \times time interaction was significant only in these tests; Table 2), although Tukey's test did not identify differences between oiling categories. The densities of nematodes and copepods were not affected by oiling (Table 2). The densities of all taxa (except *M. aestuarina*) as well as copepod species richness increased significantly over time (2-way ANOVA; Table 2), and densities were most commonly significantly lower at 18 mo compared to later time periods (Tukey's test). For example, nematode density was significantly lower at 18 than 42 mo, and polychaetes (excluding *M. aestuarina*) were lower in abundance at 18 mo compared to all other time periods. Copepod species richness was significantly lower at 18, 24, and 30 mo than at 40 and 42 mo. Effects sizes, however, were small for all tests, mostly <0.50 .

Oiling affected the meiofauna community expressed as major-taxon abundance (ANOSIM, $p = 0.001$). Reference differed from both moderately ($p = 0.003$) and heavily oiled sites ($p = 0.001$), and moderately differed from heavily oiled sites ($p = 0.035$). *M. aestuarina*, ostracods and kinorhynchs were responsible for most of the dissimilarities among the oiling intensities (*M. aestuarina* always contributed the most, about 16%, of the dissimilarities based on SIMPER). The meiofauna community also differed over time (ANOSIM, $p = 0.001$). The community at 18 mo differed from all other sampling times, the community at 24 mo also differed from those at 36, 40, and 42 mo (ANOSIM post-hoc tests; Table 3). *M. aestuarina* and polychaetes (excluding *M. aestuarina*) contributed most to dissimi-

larities among collections, each usually ranging between 10 to 15% of the total (SIMPER analysis). MDS was used to compare heavily oiled and reference meiofaunal communities at 18 vs. 24, 30 vs. 36, and 42 vs. 48 mo (see Fig. S2 in the Supplement). Heavily oiled samples at 18 mo were clearly separated from reference samples and from heavily oiled samples at 24 mo, while reference samples from 18 and 24 mo were largely interspersed. Reference samples remained clearly separated from heavily oiled samples at 30 and 36 mo; however, separation was less distinct between 42 and 48 mo.

Short-term and long-term site comparison

Short-term and long-term sites were both sampled on 2 to 3 occasions until 2.5 yr after the spill. TPH concentrations decreased more slowly and biotic recovery was slower at the heavily oiled short-term sites. Although TPH at heavily oiled short-term sites at 13 mo and long-term sites at 9 mo were similar, (450 and ~500 mg g⁻¹ respectively), TPH at the short-term sites persisted at higher levels over time (Table 1); TPH values at the heavily oiled sites averaged ~4 to 5 times higher than at short-term sites between 22 and 30 mo after the spill. *Spartina* % cover at 29 mo in the heavily oiled short-term sites was ~1% (and total vegetative cover was ~20%) compared to 87% cover by *Spartina* in lightly oiled plots, and therefore both total vegetation cover and the dominance of *Spartina* decreased with heavy oiling (Zengel et al. 2015). At the long-term sites, *Spartina* stem density in heavily oiled plots increased from almost 0 at 9 mo to 83% of that in reference sites at 30 mo. In contrast to the short-term sites, *Spartina* quickly increased in dominance in oiled sites, from ~40% of the total live plant biomass in reference to ~65% in moderately and heavily oiled sites. Meiofauna recovery in heavily oiled short-term sites was slower as well. At 24 mo the densities of several taxa at the heavily oiled long-term sites were similar to or even exceeded values in the reference sites. Specifically, total meiofauna was 12% lower, nematodes were 4.8% lower, copepods were 350% higher, and polychaetes (excluding *M. aestuarina*) were 8.3% higher in the heavily oiled samples. The number of copepod species was 6% higher in the heavily oiled sites (Figs. 2b–8b). For the same parameters and at 22 mo, values in heavily oiled plots in the short-term sites were between 56 and 75% lower compared to those in the lightly oiled plots.

DISCUSSION

Effects of oiling on benthic microalgae and meiofauna

The salt marsh benthic community in northern Barataria Bay, Louisiana as represented by benthic microalgae and meiofauna was strongly impacted by the *Deepwater Horizon* oil spill. The most harmful effects were observed in heavily oiled marshes where the concentration of TPH was very high, exceeding 450 mg g⁻¹ surface sediment. At the heavily oiled sites, we observed significantly altered algal photosynthetic pigment and meiofaunal community composition, and in the earliest collections taken after the spill, microalgal biomass and meiofauna density and diversity were unusually depressed. This trend was most prominent in samples taken 13 mo after the spill (11 mo after marsh oiling) when polychaetes and some crustacean taxa were absent and when total meiofauna, nematode, and copepod densities and copepod species richness were significantly lower (by 50 to 75%) in heavily than in lightly oiled sites. Furthermore, microalgal biomass (by >95%) and total meiofauna densities (by ~50 to 80%) at heavily oiled sites were much lower than published estimates from uncontaminated marshes in the region (Fleeger et al. 1981, Whipple et al. 1981, Fleeger & Chandler 1983, DeLaune et al. 1984, Fleeger 1985). Negative effects at lightly and moderately oiled sites were less prominent but were clearly expressed by low taxonomic diversity. In addition, *Spartina alterniflora* was examined at the same sampling sites. *Spartina* in heavily oiled marshes responded to the spill with nearly complete mortality (Lin & Mendelssohn 2012, Zengel et al. 2015). Initial recovery of *Spartina* was observed between 9 and 18 mo (Lin & Mendelssohn 2012), and it is likely that the benthic community began to recover before our initial collections (13 and 18 mo after the spill), masking an even higher or complete depopulation/mortality of the benthic community at heavily oiled sites.

Recovery of benthic microalgae and meiofauna from oiling

Population and community metrics indicated that benthic microalgae and meiofauna largely (but not completely) recovered from the spill at the long-term sites; variation for most metrics among reference and oiled stations was indistinguishable from natural variation ~36 mo after the spill. Similarly, *Spartina*

density (but not plant community composition or below-ground biomass) recovered ~36 mo after the spill (Fig. 9). Macondo oil that entered marshes was emulsified and weathered, and the pulse of oil smothered plants and benthic biota (Zengel et al. 2015) in addition to exposing biota to high levels of petroleum hydrocarbons. Recovery of vegetation, meiofauna, and benthic microalgae may have begun as the concentration of oil decreased by microbial degradation and erosion, as bioavailability decreased as oil became integrated into the structure of the soil (Chung & Alexander 1999), and/or as biota gained improved access to resources such as light, oxygen, and food associated with the soil surface. The concurrent recovery of *Spartina* may have also facilitated recovery of the benthic community (see below).

Several observations more closely associated the recovery of the meiofauna community with the recovery of *Spartina* stem density rather than with total (or complete) vegetative recovery per se. For example, *Spartina* (based on stem density) and meiofauna density both recovered before vegetative recovery (as measured by species composition and below-ground biomass) was complete at the long-term sites, and the rate of recovery of meiofauna was much slower at the short-term sites where *Spartina* recovery was also slower. Although vegetative recovery at the short- and long-term sites differed substantially, *Spartina* and meiofauna responses paralleled each other. Species other than *Spartina* partially colonized areas that suffered almost complete vegetative mortality (and the dominance of *Spartina* decreased) at the heavily oiled short-term sites (Zengel et al. 2015), and meiofauna recovered slowly. At the long-term sites, the dominance of *Spartina* quickly increased in oiled plots even though *Spartina* suffered nearly 100% initial mortality, and recovery of meiofauna was more rapid. Furthermore, the intensities of recovery were similar. At the long-term sites, the highest *Spartina* stem density, the highest copepod species richness, and the highest densities of meiofauna were consistently observed at moderately oiled sites. *Spartina* stem density from 18 to 30 mo after the spill was 1.5 to 2 times higher at moderately oiled sites compared to reference sites, and Tukey's test indicated a significantly higher density. *Spartina* has been shown to increase in biomass from sub-lethal hydrocarbon exposure (Li et al. 1990), and the increase in stem density we observed was likely either a physiological response to oiling-induced stress (Li et al. 1990) or to release from competition associated with the reduction in density of *Juncus roemerianus* caused by oiling (Lin & Mendels-

sohn 2012). At the same time, copepod species richness was higher by ~1.5 times at moderately oiled compared to reference sites, although ANOVA indicated a significant interaction between time and oiling category but not an oiling effect. A 2-tailed *t*-test was subsequently conducted to compare copepod species richness only in moderately oiled and reference sites collected from 18 to 30 mo after the spill. Species richness was significantly higher at moderately oiled than reference sites ($p = 0.045$, $df = 14$). The density of total meiofauna was also ~1.5 times higher at moderately oiled than reference sites; however, this trend was not supported by Tukey's test, as moderately oiled sites differed from heavily oiled but not reference sites. Nevertheless, the concurrent responses and equivalent intensities in the earliest stages of recovery indicate a strong link between *Spartina* and the benthic community.

Previous research has shown that *Spartina* has many positive effects on the salt marsh benthic community, and *Spartina* recovery likely facilitated benthic recovery, possibly by more than one mechanism. Craft et al. (2003) found a close correspondence between *Spartina* stem density and infaunal density and diversity in restored marshes, and Craft & Sacco (2003) found that the recovery of infauna, including *Manayunkia aestuarina*, in constructed marshes correlated with factors (e.g. bulk density and organic matter content) associated with soil quality that could be degraded by a high oil content. As *Spartina* recovered from the spill, increases in soil oxygen and changes in organic matter, for example, likely improved habitat quality at the heavily oiled sites. The physical presence and/or cover provided by *Spartina* stems also affects benthic microalgae and meiofauna. Stems baffle flow and alter critical erosion velocity affecting passive emergence into the water column, thereby influencing the sediment abundance of meiofauna (Fleeger et al. 1984, Palmer 1986). Stems also moderate soil temperature and light intensity that affect the biomass and composition of the benthic microalgal community (Whipple et al. 1981). *Spartina* stems in Louisiana also host dense epiphytic algae (Quiñones-Rivera & Fleeger 2005), and the absence of stems reduces algal and microbial abundance in the marsh surface microlayer (Seliskar & Gallagher 2014), a source of food for infauna including *M. aestuarina* (Galván et al. 2011). Finally, DeLaune et al. (1984) concluded that cutting and removing stems as a method of clean up after oil spills impacted meiofauna more than oiling without clean up.

Although the density of total meiofauna recovered ~36 mo after the spill, some taxa were not fully recov-

ered after 48 mo. The abundances of *M. aestuarina* and kinorhynchs in heavily oiled sediment remained near zero until 4 yr after the spill, when increases to ~50% of reference densities were observed. Kinorhynchs were also slow to recover in moderately oiled sediment, and ostracod abundance was near zero in heavily oiled marshes more than 2 yr after the spill. These taxa may be less tolerant of hydrocarbons or they may be dependent on the recovery of the soil environment as petroleum hydrocarbon content decreases over time. In addition, life history traits may have contributed to their slow recovery. *M. aestuarina* is a tube-dweller with direct development; juveniles develop in the mother's tube (Bell 1982). Kinorhynchs also lack dispersing larvae and are non-swimming. Low dispersal potential, compared to infauna that frequently enter the water column as adults and juveniles (Chandler & Fleeger 1983) or experience larval dispersal (Craft & Sacco 2003), may also limit recovery.

Disturbance-specialist meiofauna species are commonly reported in marine environments, especially those perturbed by organic enrichment (Gee & Warwick 1985, Warwick et al. 1988). We observed a very high abundance of the harpacticoid copepod *Harpacticus* sp. in 1 collection (at 30 mo post spill) in heavily oiled sediment, suggesting this species was able to take advantage of the recovering but still disturbed environment found there. There was a strong peak in microalgal biomass in heavily oiled sediment coincident with the abrupt dominance of *Harpacticus* sp. at 30 mo, suggesting that *Harpacticus* sp. was able to take advantage of an increased food level. Otherwise, the copepod species assemblage was very similar in oiled and reference marshes, and recovery was not characterized by changes in species composition indicative of succession after a major disturbance.

Possible oiling effects on reference sites

The observed pattern of very low abundance in the earliest collections taken after the spill suggests that our reference and lightly oiled sites were impacted by the spill, even though dominant vegetation was not obviously affected. Similarly, Pennings et al. (2014) detected short-term impacts to terrestrial arthropods and fiddler crabs in areas with minimal oiling and apparently healthy vegetation. In the early stages of recovery, low densities of several taxa (e.g. nematodes, copepods, and polychaetes) occurred in all reference and oiled sites. The similarity in

response at reference and oiled sites is reflected in the rarity of statistical interactions between oiling intensity and time among taxa (Table 2). Variation at reference marshes over time was therefore similar to variation at oiled marshes, i.e. increases in density over time occurred at all exposure levels. Interannual variation is well known to be high in meiofauna (Mittwally & Fleeger 2013), but it is unlikely that the low densities we observed can be attributed solely to natural variation. The lowest densities in many taxa simultaneously occurred at our oiled and reference sites, but peaks and valleys associated with natural variation are seldom synchronous among taxa in long-term datasets (Coull 1985). Although high levels of oil in Barataria Bay in 2010 could have induced mortality in the developing larvae of some polychaetes, and reduced recruitment and juvenile density in our earliest collections at reference sites, the most abundant meiofauna (e.g. nematodes, copepods, *M. aestuarina*) lack larval dispersal and could not have been similarly affected. Although TPH concentrations at reference sites were much lower than at oiled sites (Table 1), biota may have also been affected by exposure to poly-cyclic aromatic hydrocarbons (PAH), a highly toxic component of crude oil not quantified by our hydrocarbon analysis. Turner et al. (2014) reported that the oil spill increased sediment PAH concentrations in Louisiana wetlands by >150 times. PAH may have been elevated at our reference sites given the widespread distribution of Macondo oil in Barataria Bay, although total PAH at the lightly oiled short-term sites was low (0.083 ± 0.038 ; Zengel et al. 2015). Pollution-induced effects-at-a-distance alternatively may explain low densities at reference sites (Spromberg et al. 1998). Meiofauna, including kinorhynchs, oligochaetes, and copepods may function as metapopulations (Johnson 2001, Junkins et al. 2006, Yamasaki et al. 2014). When local metapopulations are reduced in abundance by pollution events, the resulting diminished migration to neighboring sites may reduce abundance at all sites (including reference sites) without direct exposure to the pollutant.

A full recovery of the plant community following the *Deepwater Horizon* oil spill may take 5 yr or longer in heavily oiled Louisiana salt marshes (Lin & Mendelssohn unpubl. data). Although some meiofaunal taxa proved less resilient (and the community as a whole will therefore probably take 5 yr or more to recover), high levels of primary and secondary production provided by benthic microalgae and meiofauna should return as biomass and abundance reach levels found at reference sites. These observa-

tions suggest that *Spartina* can be used as a proxy to monitor recovery of ecosystem function in salt marshes following oil spills, at least in regards to food web support at the base of the food web. However, we caution that some *Spartina* marshes may have been subjected to accelerated erosion in heavily oiled marshes in northern Barataria Bay, and the severity of marsh loss may not be fully indicated by *Spartina* stem density.

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