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Impacts of the *Deepwater Horizon* Oil Spill on Salt Marsh Periwinkles (*Littoraria irrorata*)

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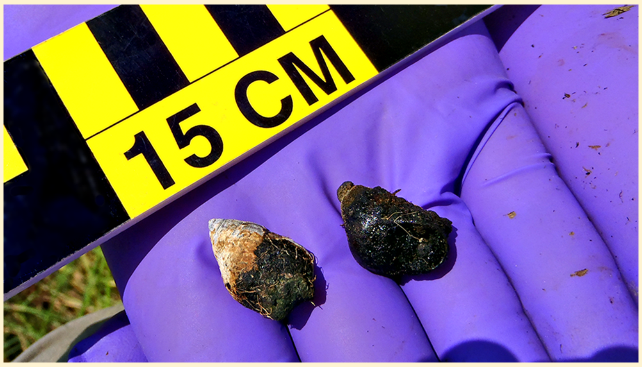
Supporting Information



ABSTRACT: *Deepwater Horizon* was the largest marine oil spill in U.S. waters, oiling large expanses of coastal wetland shorelines. We compared marsh periwinkle (*Littoraria irrorata*) density and shell length at salt marsh sites with heavy oiling to reference conditions ~16 months after oiling. We also compared periwinkle density and size among oiled sites with and without shoreline cleanup treatments. Densities of periwinkles were reduced by 80−90% at the oiled marsh edge and by 50% in the oiled marsh interior (~9 m inland) compared to reference, with greatest numerical losses of periwinkles in the marsh interior, where densities were naturally higher. Shoreline cleanup further reduced adult snail density as well as snail size. Based on the size of adult periwinkles observed coupled with age and growth information, population recovery is projected to take several years once oiling and habitat conditions in affected areas are suitable to support normal periwinkle life-history functions. Where heavily oiled marshes have experienced accelerated erosion as a result of the spill, these habitat impacts would represent additional losses of periwinkles. Losses of marsh periwinkles would likely affect other ecosystem processes and attributes, including organic matter and nutrient cycling, marsh-estuarine food chains, and multiple species that prey on periwinkles.

INTRODUCTION

The *Deepwater Horizon* incident, starting in April 2010, was the largest marine oil spill in U.S. waters and one of the largest worldwide: 3.19 million barrels of oil were released into the Gulf of Mexico[1] and over 2100 km of shoreline were oiled, including over 1100 km of coastal wetland shorelines.[2,3] As part of the Natural Resources Damage Assessment (NRDA) process, natural resource trustees are tasked with assessing resource injuries and recovery to allow identification of appropriate restoration. Here, as part of the NRDA injury assessment, we address the impact of the *Deepwater Horizon* oil spill on the marsh periwinkle, *Littoraria irrorata*, one of the most abundant and important macroinvertebrates in salt marshes of the Gulf to Mid-Atlantic Coasts of the United States.[4−9]

Marsh periwinkles are important prey for multiple species including blue crab (*Callinectes sapidus*), diamondback terrapin (*Malaclemys terrapin*), clapper rail (*Rallus longirostris*), and northern raccoon (*Procyon lotor*).[10−16] Periwinkles also have important influences on marsh vegetation, organic detritus, nutrient cycling, microbial communities, other invertebrates, and ecosystem productivity.[6,17−23] For example, periwinkle grazing plays a key role in the shredding and decomposition of senescent and dead *Spartina alterniflora* leaves, supporting organic matter and nutrient cycling and marsh-estuarine food

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chains.[17,18] Periwinkle grazing may also regulate plant productivity, and in some cases, periwinkles may respond or contribute to marsh vegetation die-back events.[6,19,24] Accordingly, oil spill impacts to periwinkles could affect a variety of other species and overall marsh function.

Impacts to salt marsh habitats and biota resulting from the *Deepwater Horizon* oil spill have been documented in previous papers.[25−30] Silliman et al.[27] and Zengel et al.[29,30] observed reduced densities of marsh periwinkles at the heavily oiled marsh edge, where the vegetation was also impacted. In contrast, McCall and Pennings[26] did not observe impacts to periwinkles in areas just inland of heavily oiled shorelines, where there was less visible oiling and the vegetation appeared unaffected. These prior studies were primarily focused on topics other than marsh periwinkles and each had either limited replication (as few as three oiled sites) or examined relatively small areas of shoreline (<1 km), and were not definitive concerning the extent or degree of marsh periwinkle impacts. A few prior studies have also documented impacts to marsh periwinkles from other oil spills, with effects including increased mortality, reduced densities, reduced recruitment, and altered size distributions.[31−34] Potential impacts to periwinkles could result from direct oiling of individuals, reductions or losses of marsh vegetation caused by oiling, and incorporation of oil into surficial marsh soils, affecting periwinkles, their habitat, or food sources. Periwinkles could be particularly affected by changes in the marsh vegetation due to their close association with *Spartina alterniflora*,[8,35,36] the defining feature of their habitat. Vegetation impacts resulting from the *Deepwater Horizon* incident have been documented in heavily oiled marshes by several investigators.[25,27,29,30,37,38] Accelerated marsh erosion due to vegetation impacts resulting from the oil spill have also been observed.[27,30,38,39] Increased erosion would reduce habitat area, quality, and stability, representing additional impacts to periwinkles.

Our study focused on oil spill impacts to marsh periwinkles at salt marsh sites with heavy oiling, based on sampling ∼16 months after heavy oiling came ashore in June 2010 in Barataria Bay, Louisiana (Supporting Information Figure S1). Because shoreline cleanup treatments were applied to many marshes with heavy oiling in an attempt to minimize damage and foster habitat recovery, though at some risk of causing additional impacts, we also examined the effects of operational-scale shoreline treatments on periwinkles (as opposed to prior research on smaller-scale treatment tests[29,30]). We consider the present work the definitive study to date on marsh periwinkle impacts following the *Deepwater Horizon* oil spill, due to (a) the larger number of oiled study sites and geographic area addressed compared to prior studies (24 oiled sites; ∼ 70 km of oiled marsh shorelines); (b) the examination of both oiling and operational-scale cleanup treatment effects; (c) the simultaneous sampling of both the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of the main oiling bands; and (d) the combined consideration of periwinkle density, size, population structure, and life-history stage. In addition to the presentation of our findings, we also synthesize information across this and prior *Deepwater Horizon* studies touching on marsh periwinkles.

Our main hypothesis was that marsh periwinkle densities would be reduced in locations with heavy oiling relative to reference conditions, due to oiling of periwinkles and impacts to the vegetation. If periwinkle densities were reduced as a result of oiling, their populations could also be shifted toward smaller (younger) individuals recruited after the spill. Therefore, we also hypothesized that mean snail size would be reduced in locations with heavy oiling relative to reference sites, affecting periwinkle size distributions. Oiled marsh cleanup involves difficult balancing among: speeding the removal and degradation of oil, enhancing habitat recovery, and not causing further damage to the habitat or biota.[30] Accordingly, we hypothesized that operational-scale cleanup treatments would have an effect on marsh periwinkles, though we were uncertain concerning the direction of effects, as they could be positive or negative depending on the effects of oiling, the effectiveness of treatments, and physical disturbance to the snails, the vegetation, and substrate. However, given the nature of the treatments (described below in the Materials and Methods section), we anticipated that treatment could have negative effects on periwinkles, at least over the short-term.

■ MATERIALS AND METHODS

Study Sites. Study sites were located in mainland herbaceous salt marshes in Louisiana dominated by *Spartina alterniflora*, stratified by oiling conditions and shoreline treatment (Figure S1). Our study sites were a subset of a larger NRDA coastal wetland vegetation study,[37] with our sites focused on reference conditions versus heavy initial oiling, based on Shoreline Cleanup Assessment Technique (SCAT) and NRDA shoreline oiling surveys. The vast majority of our oiled sites had heavy maximum SCAT oiling, with heavy oiling persisting on the shoreline for 3 months or longer.[2,3] Initial heavy oiling conditions in June 2010 involved thick deposits of emulsified oil on the marsh vegetation and substrate (Figure S2a).[3,40] Our oiled sites each had 90−100% vertical oil coverage on the aboveground vegetation and oiling of marsh soils, based on site-specific NRDA surveys[37] and SCAT observations.[3,40] Reference sites had no visible oiling documented during a series of site-specific NRDA surveys.[37] We omitted four original study sites with inconsistent oiling histories which could not be reliably classified as reference or heavy oiling; we also omitted two sites that were located in brackish marsh rather than salt marsh.

Oiled sites consisted of two groups. The first group included sites with heavy oiling that were randomly selected and established prior to the onset of oiled marsh shoreline treatments (as were the reference sites).[37] These oiled sites were designated as no treatment "set-asides" by the *Deepwater Horizon* Unified Command (UC; consisting of the U.S. Coast Guard, state governments, and BP). Shoreline treatments were not applied in these sites and we classified these as "oiled and untreated". All of these oiled sites had confirmed forensic matches to *Deepwater Horizon* oil based on prior NRDA sampling. The remaining oiled sites were randomly established in shoreline areas designated for treatment under the main Shoreline Treatment Recommendation (STR) issued by the UC for Barataria Bay salt marshes (STR S3-045[30,40]). We classified these sites as "oiled and treated" (with the exception of two sites that were confirmed as not treated, which we reassigned to the oiled and untreated group). Treatments included combinations of manual and mechanical removal of oiled wrack and oiled vegetation mats, cutting and raking of vegetation, raking and scraping of thick (>1 cm) oil deposits from the marsh substrate, and application of loose organic sorbents.[30,40] Most treated sites included the full suite of intensive treatments described above. Treatments were conducted between February and August 2011.

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In total, we studied 11 reference sites, 12 oiled and untreated sites, and 12 oiled and treated sites (24 oiled sites, 35 total sites). Reference sites spanned the Barataria-Terrebonne Estuary in Louisiana; oiled sites were located in Barataria Bay (Figure S1).

Sampling Methods. We sampled marsh periwinkles, marsh vegetation, and surface oiling parameters on 16−31 October 2011, ~16 months after heavy oiling initially came ashore. For each sampling site, a linear transect was established perpendicular to the shoreline extending from the seaward marsh edge into the marsh interior (Figure S3). Two 0.25 m^2 quadrats were located at each of three distances (sampling zones) from the shoreline: Zone 1, seaward marsh edge (quadrats centered an average of 2 m from the shoreline, defined by the erosional scarp at the margin of the marsh platform); Zone 2, the oiled marsh interior, the center of the maximum initial oiling width at each site (quadrats centered an average of 9 m from the shoreline, oiling width defined as the inland extent of visible oiling perpendicular to the shoreline); and Zone 3, the marsh interior located 3 m landward of the maximum initial oiling width at each site (quadrats centered an average of 21 m from the shoreline). Designations for the marsh edge versus the marsh interior were similar to Peterson and Turner.[41] For the oiled sites, Zones 1 and 2 were located within the original footprint of visible oiling at each site, as documented during prior NRDA surveys, whereas Zone 3 was located beyond (inland of) the original oiling footprint. Sampling zones in the reference sites were positioned to mirror the sampling zones in the oiled sites, based on the initial oiling widths observed during prior NRDA surveys. Data from the two quadrats in each zone at each site were averaged and converted to 1 m^2 basis prior to analyses. Zone 1 quadrats could not be effectively sampled at three sites (two reference and one oiled and untreated) due to shoreline erosion or disturbance from human-made debris on the shoreline; however, Zones 2 and 3 were sampled at these locations.

We collected all marsh periwinkles visible on the vegetation and marsh substrate within each quadrat to determine mean total density and shell length. We did not search for smaller juvenile (<6 mm shell length) periwinkles hidden between the leaf sheath and the stem of *Spartina alterniflora* shoots, or in rolled up senescent or dead leaves (the microhabitats where the smallest snails occur but where larger snails are not found), though we did sample juvenile snails visible on the vegetation or substrate. Thus, juvenile snails were likely under-sampled in our study, as they are in most similar studies. Although under-sampling of small juveniles could affect our size comparisons to some degree, the size ranges and life-stages collected (including larger juveniles, subadults, and adults) were still sufficient to examine our hypotheses given the time since the spill occurred (see the following Data Analysis subsection). Periwinkle shell lengths were measured to the nearest 0.5 mm using calipers. Periwinkles were returned to the quadrats from which they were collected at the completion of sampling. Supporting data collected for each quadrat included residual surface oil cover (%), surface oil thickness (cm), and surface oil character (e.g., emulsified oil, oil residue) based on standard shoreline assessment methods used during spills.[42] *Spartina alterniflora* live cover (%) was visually estimated for each quadrat as a numerical value (rather than as cover ranks or classes). Vegetation height (cm) was measured as "canopy height" based on the predominant height of vegetation within each quadrat.

Marsh soils were sampled for total polycyclic aromatic hydrocarbons (tPAH) during September−November 2010 and 2011 as part of a related NRDA sampling program. Marsh soil sampling areas were laterally offset by ~20 m from the marsh periwinkle quadrats to avoid interference between multiple NRDA sampling programs that were concurrently underway at the same study sites (the NRDA marsh study sites were defined as 50 m lengths of shoreline with similar oiling conditions). Soil samples were collected by hand from the top 2 cm of marsh soils directly into clean glass jars. TPAH in marsh soils was determined using GC/MS-SIM (gas chromatography/mass spectrometry in selective ion monitoring mode) based on EPA Method 8270D. TPAH included the sum of 54 PAHs, including alkylated homologues, presented as mg/kg. Marsh soils were sampled for tPAH in Zones 1 and 2; no samples were collected in Zone 3 as defined in our study.

Data Analysis. Our sampling design included treated and untreated oiled sites as well as reference sites. The objective of our study was to assess the effects of both oiling and treatment; therefore, the subsequent analyses were conducted as ANOVA planned comparisons, also known as planned contrasts or a priori comparisons. Planned comparisons are statistically powerful tests which allow researchers to answer specific questions of interest by focusing on subsets of interest.[43] Unlike post hoc pairwise comparisons, planned comparisons of independent subsets do not require any correction of *p*-values.[43] Furthermore, planned comparisons directly incorporate the ANOVA within-group mean square errors, and thus are superior to separate two-sample *t* tests.[44] In this work, two types of planned comparisons were conducted: (a) comparisons of oiled versus reference sites to assess the effects of oiling on periwinkles and supporting metrics; and (b) comparisons of treated and untreated sites to assess the effects of cleanup treatments on periwinkles and supporting measures in oiled areas.

As part of the planned comparisons, Levene's test was used to examine homogeneity of variance; when variances were unequal, Welch's methodology for generalization of two-sample tests was applied.[45] One-tailed tests were used where the direction of difference was predicted prior to testing. Two-tailed tests were used where the direction of difference was not predicted. We defined statistical significance as $p \leq 0.10$, based on guidance regarding balancing Type I and II errors during environmental impact assessments, according to our sample size.[46] Finally, for each planned comparison, the effect size was calculated using Cohen's *d* statistic.[47] Effect size thresholds were $d = 0.2$ (small effect), 0.5 (medium effect), and 0.8 (large effect) regardless of direction (i.e., sign).[47,48]

Size-frequency histograms were generated to further examine variation in periwinkle shell length. Life-history stages were incorporated into the histograms based on shell length ranges, with individuals <6, 6−13, and >13 mm in length defined as juveniles, subadults, and adults, respectively.[8,49,50] Where we observed differences in size-frequency distributions, we used Kolmogorov−Smirnov tests to determine if such differences were statistically significant. Age and growth information from the literature[51,52] was used to approximate periwinkle age according to shell size, assuming similar growth rates. Based on this, juvenile snails would be <1 year in age, subadult snails would be <1−1.5 years, and adult snails would be >1.5 years in age. Considering that most periwinkles present when the oil came ashore in 2010 would typically be in the adult size range

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Table 1. Marsh Oiling and Vegetation Characteristics[a]

		reference		oiled untreated		oiled treated					
parameter	zone	mean ± SE	*N*	mean ± SE	*N*	mean ± SE	*N*	*p*-value reference v. oiled	effect size reference v. oiled	*p*-value untreated v. treated	effect size untreated v. treated
surface oil cover (%)	1	0 ± 0	9	20 ± 10	11	19 ± 7	12	0.002	−1.7	0.919	0.1
surface oil cover (%)	2	0 ± 0	11	0.1 ± 0.1	12	0.3 ± 0.3	12	0.199	−0.3	0.451	−0.3
surface oil cover (%)	3	0 ± 0	11	0 ± 0	12	4 ± 4	12	0.323	−0.7	0.323	−0.6
2010 soil tPAH (mg/kg)	1	0.4 ± 0.1	9	312 ± 115	8	no data		0.015	−2.1		
2010 soil tPAH (mg/kg)	2	1 ± 0.4	11	25 ± 9	9	no data		0.016	−1.8		
2010 soil tPAH (mg/kg)	3	no data		no data		no data					
2011 soil tPAH (mg/kg)	1	0.5 ± 0.1	8	157 ± 49	7	65 ± 33	8	0.002	−2.5	0.146	1.0
2011 soil tPAH (mg/kg)	2	0.4 ± 0.1	11	23 ± 5	11	40 ± 19	11	0.005	−1.9	0.428	−0.5
2011 soil tPAH (mg/kg)	3	no data		no data		no data					
Spartina alt. cover (%)	1	54 ± 7	9	29 ± 8	11	25 ± 8	12	0.005	1.1	0.690	0.2
Spartina alt. cover (%)	2	54 ± 8	11	32 ± 9	12	31 ± 8	12	0.023	0.8	0.939	0.0
Spartina alt. cover (%)	3	55 ± 7	11	47 ± 8	12	42 ± 7	12	0.272	0.4	0.563	0.2
vegetation height (cm)	1	54 ± 4	9	51 ± 11	11	26 ± 7	12	0.022	1.0	0.078	0.9
vegetation height (cm)	2	54 ± 6	11	49 ± 7	12	42 ± 6	12	0.140	0.4	0.446	0.3
vegetation height (cm)	3	59 ± 5	11	59 ± 4	12	51 ± 6	12	0.603	0.2	0.299	0.4

[a]Data are means ±1 standard error (SE) with *N* number of replicates. Zones 1, 2, and 3 refer to the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of the initial maximum oiling extent, respectively. *P*-values are provided for ANOVA planned comparisons of reference versus oiled sites, and untreated versus treated sites (oiled sites with and without marsh cleanup treatments) by zone. Effect sizes based on Cohen's *d* statistic are provided for each planned comparison. Effect size thresholds are $d = 0.2$ (small effect), 0.5 (medium effect), and 0.8 (large effect), regardless of sign.

at the time we sampled in 2011 (1.3−1.4 years later), we also examined adult periwinkle density.

RESULTS

Oiling Conditions at the Time of Sampling. Surface oil cover was zero in all zones at the reference sites (Table 1). In contrast, mean surface oil cover for the oiled sites was 19−20% in Zone 1 (a large effect size) (Table 1, Figure S2b). The oil character observed in Zone 1 for the oiled sites was predominantly surface oil residue (semicohesive heavily oiled surface soils). Oil thickness was primarily in the thickness class of >0.1−1 cm thick. Zone 2 quadrats had <1% surface oil cover in the oiled sites. A few of the oiled and treated sites also had observations of oil in Zone 3, mainly as oil sheen or film. Within the oiled sites, there were no differences in surface oil cover between the untreated and treated sites in any zone. Mean 2010 and 2011 tPAH values in marsh soils were ≤1 mg/kg for the reference sites and 25−300 times higher for the oiled sites in Zones 1 and 2 (large effect sizes for both zones in both years) (Table 1). Although not statistically significant, the effect size for tPAH in Zone 1 for untreated versus treated sites was also large, treated sites tending to have lower tPAH than untreated sites.

Vegetation Conditions at the Time of Sampling. Mean *Spartina alterniflora* live cover values at the reference sites were 54−55% across all three zones (Table 1). *Spartina alterniflora* live cover was reduced by 40−50% in Zone 1 and 2 at the oiled versus reference sites (large effect sizes in both zones), but did not greatly differ between oiled and reference in Zone 3. *Spartina alterniflora* cover did not differ between the untreated and treated oiled sites in any zone. Mean vegetation heights at reference sites were 54−59 cm across all three zones (Table 1). Mean vegetation height was reduced to 51 cm for the oiled and untreated sites in Zone 1, and was further reduced to 26 cm for the oiled and treated sites in Zone 1 (effect sizes were large for both reference versus oiled and for untreated versus treated sites). Vegetation height did not greatly differ in Zones 2 or 3.

Marsh Periwinkles. Marsh periwinkle densities at reference sites increased from a mean of 34 snails m^{-2} in Zone 1 to means of 80 or more snails m^{-2} in Zones 2 and 3, in close agreement with reported periwinkle densities for Louisiana salt marshes (Figure 1, Table 2). Periwinkle densities in the oiled sites were reduced by 80−90% from reference values in Zone 1 and were reduced by 50% in Zone 2 (effect sizes were large in both zones). Densities in Zone 3 did not differ between reference and oiled sites. Among the oiled sites, densities in the treated sites were nearly 60% lower than in the untreated sites in Zone 1; although this difference was not statistically significant, the effect size was large (Figure 1).

Periwinkle mean shell length ranged from 17 to 21 mm across most combinations of oiling/treatment classification and zone (Figure 2). Shell length did not differ between oiled and

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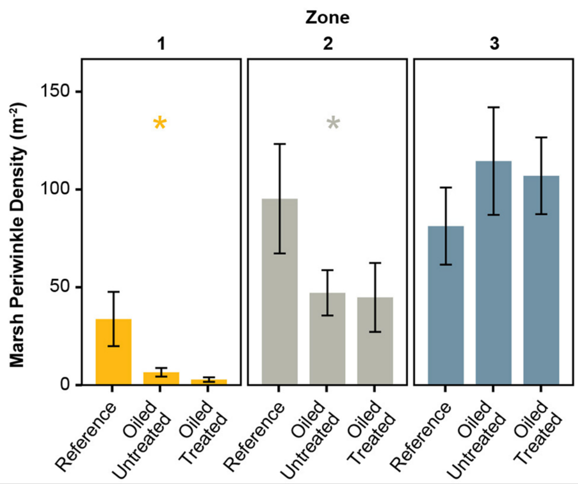
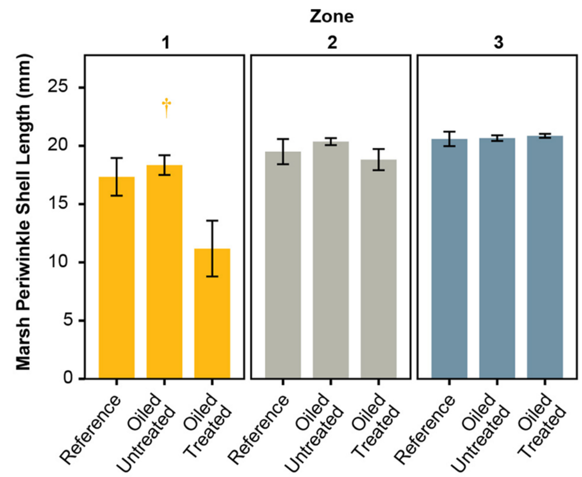


Zone	Reference Mean ± SE	N	Oiled Untreated Mean ± SE	N	Oiled Treated Mean ± SE	N	p-value ref v. oiled	effect size ref v. oiled	p-value untreated v. treated	effect size untreated v. treated
1	34 ± 14	9	7 ± 2	11	3 ± 1	12	0.035	1.6	0.155	0.8
2	95 ± 28	11	47 ± 12	12	45 ± 18	12	0.062	1.0	0.913	0.1
3	81 ± 20	11	115 ± 27	12	107 ± 20	12	0.305	-0.4	0.815	0.1

Figure 1. Marsh periwinkle total densities. Data are means ±1 standard error (SE) with *N* number of replicates. Zones 1, 2, and 3 refer to the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of the initial maximum oiling extent, respectively. *P*-values are provided for ANOVA planned comparisons of reference versus oiled sites, and untreated versus treated sites (oiled sites with and without marsh cleanup treatments) by zone. *Indicates planned comparisons of reference and oiled sites were statistically significant at $p \leq 0.10$. Effect sizes based on Cohen's *d* statistic are provided for each planned comparison. Effect size thresholds are $d = 0.2$ (small effect), 0.5 (medium effect), and 0.8 (large effect), regardless of sign.



Zone	Reference Mean ± SE	N	Oiled Untreated Mean ± SE	N	Oiled Treated Mean ± SE	N	p-value ref v. oiled	effect size ref v. oiled	p-value untreated v. treated	effect size untreated v. treated
1	17.3 ± 1.6	9	18.3 ± 0.8	6	11.2 ± 2.4	7	0.119	0.6	0.016	1.2
2	19.5 ± 1.1	11	20.4 ± 0.3	11	18.8 ± 0.9	9	0.465	0.0	0.205	0.5
3	20.6 ± 0.6	11	20.7 ± 0.2	12	20.9 ± 0.2	11	0.799	-0.2	0.508	-0.3

Figure 2. Marsh periwinkle shell length. Data are means ±1 standard error (SE) with *N* number of replicates. Zones 1, 2, and 3 refer to the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of the initial maximum oiling extent, respectively. *P*-values are provided for ANOVA planned comparisons of reference versus oiled sites, and untreated versus treated sites (oiled sites with and without marsh cleanup treatments) by zone. †Indicates planned comparisons of untreated and treated sites were statistically significant at $p \leq 0.10$. Effect sizes based on Cohen's *d* statistic are provided for each planned comparison. Effect size thresholds are $d = 0.2$ (small effect), 0.5 (medium effect), and 0.8 (large effect), regardless of sign.

Table 2. Reported Marsh Periwinkle Densities from Louisiana Salt Marshes[a]

location	periwinkle density	source
Barataria Bay	24 m^{-2}	Alexander 1979[53]
Port Fourchon	68−82 m^{-2}	Silliman et al. 2005[19]
Barataria Bay	31−34 m^{-2}	Spicer 2007[54]
Sabine NWR to Bay St. Louis, MS	∼ 40−70 m^{-2}	McCall and Pennings 2012[26]
Barataria Bay	∼ 50 m^{-2}	Silliman et al. 2012[27]
Bayou LaFourche	133 m^{-2}	Stagg and Mendelssohn 2012[8]
Bayou LaFourche	34 m^{-2}	Tong et al. 2013[55]
Barataria Bay	37−183 m^{-2}	Zengel et al. 2014, 2015[29,30]
Port Fourchon	27−64 m^{-2}	McFarlin et al. 2015[36]

[a]Lower values typically from the marsh edge; higher values typically from the marsh interior or including smaller juveniles in addition to larger snails. Approximate values estimated from figures. Limited to reference sites for impact studies.

reference sites as a whole; however, in Zone 1 there was a sharp reduction in shell length to 11 mm at oiled sites that were treated (effect size was large for untreated versus treated, and medium for oiled versus reference sites). Periwinkle shell length−frequency histograms revealed that adult snails were predominant (mean adult shell length 21 mm), with fewer juveniles and subadults observed (Figure 3). The exception was in Zone 1 of the oiled and treated sites (dashed red circle in Figure 3), where periwinkle populations lacked adults and consisted mainly of subadults (though in low numbers). The oiled and treated sites in Zone 2 were also partly shifted toward smaller snails (compared to the reference and untreated sites), with a bimodal distribution consisting of peaks in both the subadult and adult categories.

Analysis of adult periwinkle densities gave results similar to the analysis of total density, although Zone 1 differences between treated and untreated sites were also statistically significant (Figure 4). Adult snail densities were reduced at oiled versus reference sites by 50−90% in Zones 1 and 2 (large effect sizes in both zones). In addition, adult snail density was 80% lower in Zone 1 in the oiled and treated sites compared to the untreated sites (also a large effect size).

Geographic Versus Oiling Influences. To confirm that the multiple differences observed between the reference and oiled sites in Zones 1 and 2 were not driven by geography rather than oiling (due to the wider geographic spread of reference sites relative to oiled sites concentrated in Barataria Bay, Figure S1), we compared all measured parameters between reference sites in Terrebonne Bay and Barataria Bay. There were no differences in oiling parameters, vegetation metrics, total periwinkle density, or adult periwinkle density among bays (all $p > 0.10$). There was a difference in mean shell length in Zone 1 ($p = 0.033$). There was a smaller difference in shell length in Zone 2, although this was not statistically significant ($p = 0.158$). Snails were smaller in the reference sites in Terrebonne versus Barataria Bay, although mean shell length corresponded to the adult size range and adult snails were predominant in both bays (though dominated by adults, Terrebonne had a larger proportion of juveniles and subadults

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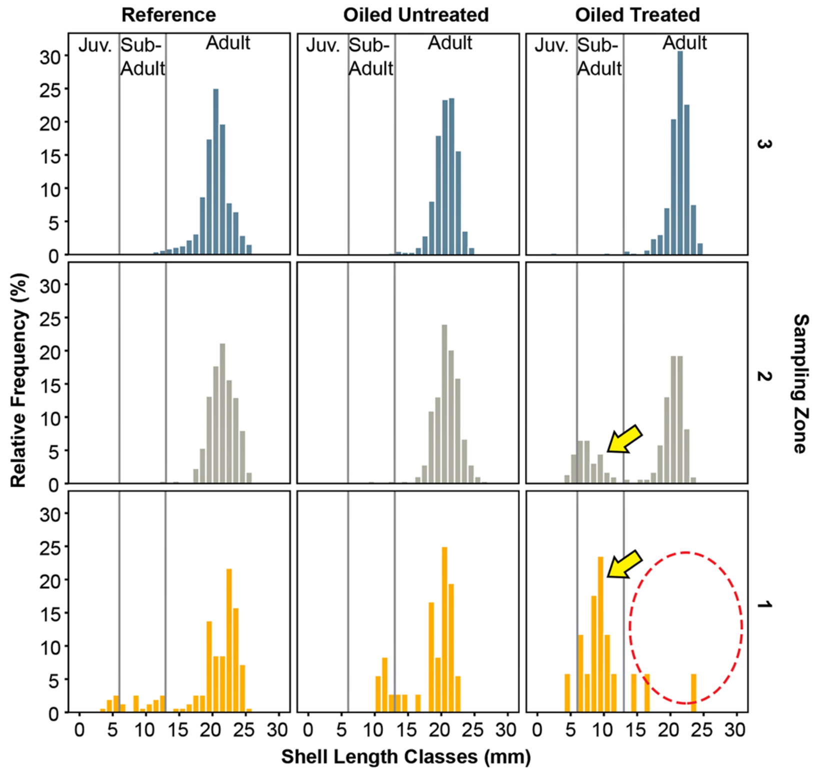

Figure 3. Marsh periwinkle shell length histograms. Periwinkle life stage classes were defined as juveniles (<6 mm), subadults (6−13 mm), and adults (>13 mm). Zones 1, 2, and 3 refer to the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of the initial maximum oiling extent, respectively. For the oiled and treated sites, the red dashed circle highlights the lack of adult snails and the yellow arrows indicate relative shifts to smaller subadult snails in Zones 1 and 2, as compared to the reference and oiled and untreated sites. Kolmogorov−Smirnov tests indicated that these differences in distributions were statistically significant in both zones (all $p < 0.001$).

in Zone 1 compared to Barataria). Geographical differences between the reference and oiled sites did not contribute to the oiling differences reported above (prior subsections). Instead, the smaller snails in the Terrebonne Bay reference sites may have limited the ability to detect some underlying differences in snail size between oiled and reference sites in Barataria. Reanalyzing mean shell length without the Terrebonne reference sites indicated that mean shell length in Zone 1 was smaller for the oiled sites (14.5 mm ±1.7 SE) versus the Barataria reference sites (20.6 mm ±1.0 SE) ($p = 0.011$, effect size large). Mean shell length in Zone 2 was also smaller for the oiled sites (19.7 mm ±0.5 SE) compared to the Barataria reference sites (21.2 mm ±0.5 SE) (p = 0.020, effect size large).

■ DISCUSSION

Sampling was conducted ∼16 months after heavy oiling initially came ashore, but our results indicated strong oiling effects on marsh periwinkles and their vegetated habitat, along with continued oil presence. We also found that treatment of oiled sites had negative effects on snails, at least over the short-term. As we discuss below, we project that recovery of periwinkle populations is likely to take multiple years.

The oiling and vegetation conditions observed at oiled sites were consistent with previous studies of the *Deepwater Horizon* oil spill.[3,25,27,29,30,37,38,56] We found residual oil present both on the marsh surface and in marsh soils. TPAH concentrations in the oiled sites were large multiples of those found at reference sites, during both 2010 and 2011, suggesting a long time frame for recovery to reference conditions.[56] In addition, we observed reductions in the cover and height of *Spartina alterniflora* at oiled sites, representing important changes in habitat characteristics for marsh periwinkles.

Marsh periwinkles were strongly affected by oiling and associated habitat alterations in our study, as evidenced by the 80−90% reduction in total periwinkle densities at the oiled marsh edge (Zone 1) and the 50% reduction in total densities in the oiled marsh interior (Zone 2). The very low snail densities at the oiled marsh edge (3−7 snails m^{-2}) were particularly striking compared to the typical range of periwinkle densities reported for Louisiana salt marshes (Table 2). Nevertheless, because periwinkle densities were higher in the marsh interior than at the marsh edge, the numerical loss of periwinkles in the oiled marsh interior (49 snails m^{-2} ± 32 SE lost to oiling) was greater in comparison to the oiled marsh edge (29 snails m^{-2} ± 14 SE lost to oiling), even though the percent reduction was higher at the marsh edge. In addition, there was some indication that periwinkle size was reduced in the oiled sites, also due to the loss of snails (the loss of larger adult snails affecting snail size, especially in the oiled and treated sites), though reduced snail growth could have also been a contributing factor.

We did not investigate the mechanisms by which oiling reduced snail densities within the marsh, but it likely included several different pathways. First, it is likely that heavy oiling directly killed periwinkles through physical smothering by thick emulsified oil, and perhaps through toxic effects as well. In a follow-on to our study, periwinkles exposed to *Deepwater Horizon* oiling conditions comparable to our study sites experienced high mortality rates following relatively short exposure durations, and did not move even short distances to

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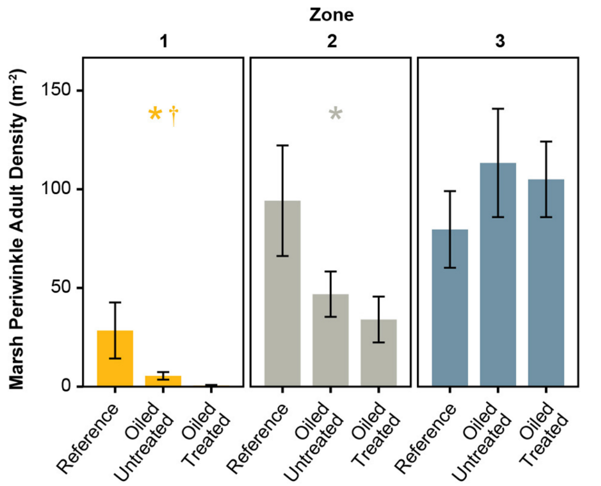

Zone	Reference		Oiled Untreated		Oiled Treated		p-value ref v. oiled	effect size ref v. oiled	p-value untreated v. treated	effect size untreated v. treated
	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N				
1	28 ± 14	9	5 ± 2	11	1 ± 0.4	12	0.056	1.4	0.027	1.6
2	94 ± 28	11	47 ± 11	12	34 ± 12	12	0.045	1.2	0.441	0.3
3	80 ± 19	11	113 ± 27	12	105 ± 19	12	0.300	-0.4	0.792	0.1

Figure 4. Marsh periwinkle adult densities. Adults were defined as snails >13 mm in shell length. Data are means ±1 standard error (SE) with *N* number of replicates. Zones 1, 2, and 3 refer to the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of the initial maximum oiling extent, respectively. *P*-values are provided for ANOVA planned comparisons of reference versus oiled sites, and untreated versus treated sites (oiled sites with and without marsh cleanup treatments) by zone. * Indicates planned comparisons of reference and oiled sites were statistically significant at $p \leq 0.10$. † Indicates planned comparisons of untreated and treated sites were statistically significant at $p \leq 0.10$. Effect sizes based on Cohen's d statistic are provided for each planned comparison. Effect size thresholds are $d = 0.2$ (small effect), 0.5 (medium effect), and 0.8 (large effect), regardless of sign.

reach unoiled vegetation and substrate.[57] Second, the effects of oiling on the vegetation removed or reduced primary periwinkle habitat. In particular, low *Spartina alterniflora* cover observed at the time of sampling likely limited periwinkle recovery. Third, residual oiling on the marsh substrate and elevated tPAH levels in surficial marsh soils could have had negative effects on periwinkles, including direct toxicity and reduced periwinkle food resources. Periwinkles spend time on the marsh substrate, where they would be exposed to residual oiling. Though we do not have information related to soil exposure concentrations that induce toxicity in periwinkles, tPAH levels observed at our oiled study sites were easily high enough to cause adverse effects in estuarine invertebrates.[58,59] Moreover, periwinkles include microalgae on the marsh substrate and in shallow marsh soils as part of their diet, and these microalgae communities showed reduced biomass and altered species composition in heavily oiled sites during and beyond our study period.[60] If *Spartina alterniflora* takes up PAHs from soils, periwinkle grazing on plant surfaces could be another exposure pathway.[61] Reduced levels of fungal food resources for periwinkles were also recorded for *Spartina alterniflora* leaf tissues in heavily oiled areas three years following initial oiling.[62] Food limitations in the marsh canopy would also negatively affect periwinkle recovery.

Shoreline treatment of oiled sites appeared to have secondary negative effects on periwinkles, including the near absence of adult snails at the marsh edge in treated sites (expressed as lower adult density and smaller snail size). Prior *Deepwater Horizon* studies looking at test-scale shoreline treatments found no periwinkle differences among oiled plots with and without treatment, though this was possibly due to large oiling effects obscuring treatment influences.[29,30] In the current study, treatments such as vegetation and substrate raking, cutting, and scraping may have directly removed, buried, or physically damaged periwinkles; exposed or re-exposed them to residual oiling; and further altered their habitat. Although our data suggest that treatment of oiled shorelines had negative effects on periwinkles, some studies have shown that shoreline treatment can benefit other marsh attributes, including the vegetation.[29,30] Shoreline treatment could eventually benefit periwinkles by removing oil and enhancing its degradation (tPAH tended to be lower in Zone 1 in our treated sites), and improving vegetation recovery, despite having negative short-term effects. Longer-term monitoring would be necessary to evaluate this possibility.

We propose that a definition of recovery for this species include attaining both densities and size distributions similar to reference conditions. One way to approach this would be to consider the time it would take to re-establish adult components of the population following successful recruitment. Based on the mean size of adult periwinkles in our study (21 mm shell length) and periwinkle age and growth information,[51,52] assuming similar growth rates (or even somewhat faster growth rates in Louisiana), we project that periwinkle population recovery will likely take at least 3−5 years once oiling and habitat conditions are suitable to support normal levels of periwinkle recruitment, immigration, survival, and growth in impacted areas (e.g., 3−5 years would be the approximate age of mean-sized adult snails). If multiple years of normal recruitment are needed to re-establish periwinkle densities and size distributions (due to high spatiotemporal variation in recruitment, low survival, high predation, etc.), recovery could take even longer. Marsh periwinkles can live to ages of at least 13 years.[51] If starting from new recruits, the time required to achieve a population structure including the largest/oldest snails could take 10 years or longer. Longer-term monitoring would be useful to examine recovery times following this spill.

Several studies examining the *Deepwater Horizon* oil spill reported marsh periwinkle results that integrate well with our larger study. Silliman et al.[27] reported 98% reductions of periwinkles from the heavily oiled marsh edge at three sites during October 2010, ~4 months following oil coming ashore. These results represent the earliest evidence of periwinkle impacts and are consistent with our Zone 1 findings. Zengel et al.[29,30] documented reductions in marsh periwinkle densities of 95−100% in heavily oiled treatment test plots during 2011−2013, extending the duration of impacts shown in our results to greater than three years following oiling. Zengel et al.[30] stated that recruitment or survival of juvenile periwinkles appeared to be lacking in their oiled plots compared to reference, due to reduced *Spartina alterniflora* cover, residual oiling levels, or both factors. Zengel et al.[29] saw possible signs of initial periwinkle recovery in plots where *Spartina alterniflora* cover had been restored (via planting), but periwinkle densities were still low, suggesting that periwinkle recovery will lag vegetation recovery. Similar lags in the recovery or development of periwinkle density or size structure relative to vegetation recovery have been observed following other oil spills, physical marsh impacts, and marsh restoration projects.[33,34,63−65]

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McCall and Pennings[26] sampled marsh periwinkle density just inland of heavy oiling bands, where *Spartina alterniflora* vegetation was intact and oiling was relatively minimal. Their sampling, conducted in 2010 and 2011, found no differences in periwinkle density between reference and oiled sites. This finding corresponds well with our results from Zone 3, where we also saw no differences in periwinkle density or size. In sum, our findings combined with those of others indicate strong impacts of heavy oiling on periwinkles, especially in areas where oiling damaged the vegetation, but no obvious impacts inland of the heavy oiling bands.

Overall, taking into account both our study and others, we conclude that substantial losses of marsh periwinkles occurred in areas with heavy oiling resulting from the *Deepwater Horizon* oil spill. New findings presented here include: (a) periwinkle impacts spanning a much larger number of sites and greater shoreline area than previously reported; (b) periwinkle impacts extending into the oiled marsh interior, where numerical losses were actually higher than at the oiled marsh edge; (c) additional negative effects of oil cleanup treatments on periwinkles; and (d) impacts including not only periwinkle density but also periwinkle size and population structure. Moreover, the impacts we observed were ongoing, continuing more than a year after heavy oiling came ashore, with few signs of recovery. Other studies have found that impacts in some locations were ongoing more than three years after heavy oiling, and that periwinkle recovery may lag recovery of the marsh vegetation. Based on these combined findings, and on periwinkle age and growth, we project that recovery of periwinkle populations is likely to take several years once oiling and habitat conditions in affected areas are suitable to support normal periwinkle life-history functions. Furthermore, although not examined in our study, where heavily oiled marsh areas have experienced accelerated erosion as a result of the spill, such habitat impacts would represent additional losses of periwinkles. In turn, the loss of marsh periwinkles would likely affect other ecosystem processes and attributes, including organic matter and nutrient cycling, marsh-estuarine food chains, and multiple species that prey on periwinkles.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04371.

Figures depicting sampling site locations (map, Figure S1), oiling conditions (photographs, Figure S2), and sampling transect and quadrat layout (Figure S3) (PDF)

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Notes

The authors declare no competing financial interest.

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