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## THEME SECTION

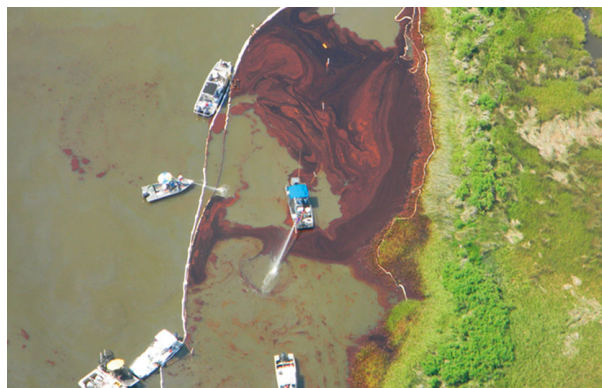
# Response of nearshore ecosystems to the *Deepwater Horizon* oil spill

Editors: Charles H. Peterson, Sean P. Powers, Just Cebrian, Kenneth L. Heck Jr.

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This Theme Section details the effects from the *Deepwater Horizon* oil spill on several critical habitats and trophic levels within nearshore coastal ecosystems. The Theme Section represents an important study of the response of a nearshore ecosystem to an oil spill and the associated response and clean-up activities.

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## INTRODUCTION

# Response of nearshore ecosystems to the Deepwater Horizon oil spill

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**ABSTRACT:** Nearshore coastal ecosystems are among the most productive environments on the planet but are threatened as a result of sea level rise, human development and pollution. These ecosystems often act as a sink for contaminants released into the open ocean as documented during the 2010 *Deepwater Horizon* oil spill. The geographic extent (1000s of km of potentially impacted beaches and marshes) and duration (87 d of oil release) of this oil spill as well as the related response and clean-up activities were unprecedented. Six years after the spill, studies supported by the *Deepwater Horizon* Natural Resources Damage Assessment as well as other independent investigations have elucidated many of the consequences of one of the world's largest oil spills. Understanding these impacts required the integration of multiple marine disciplines (e.g. physical oceanography, zoology, botany, toxicology, geospatial analysis and modeling). The contributions to this Theme Section highlight 4 key findings that are critical in assessing and responding to future oil spills: (1) organismal level effects were documented across the full range of trophic levels in areas that experienced heavy oiling; (2) degradation or loss of habitat-forming species represents a pathway to long-term direct and indirect effects; (3) the loss and degradation of these habitats result in a wide range of ecosystem service losses; and (4) response actions designed to mitigate the effects of oil often result in ecological injury. Assessment of future oil spill damages should adopt a conceptual model of injury pathways early in the impact assessment process, and this model should focus heavily on habitat-forming species.

**KEY WORDS:** Natural Resource Damage Assessment · NRDA · Saltmarsh · *Deepwater Horizon* oil spill · Injury · Environmental impact

### *Deepwater Horizon* oil spill and its effects

The *Deepwater Horizon* oil spill (DWHOS) was the largest oil spill in US history and one of the largest in the world (Fig. 1), with an immense geographic footprint (1000s of km of shoreline oiled; Rouhani et al. 2017, this Theme Section) and temporal scale (oil release for 87 d), and it challenged marine ecologists in designing assessments of its impacts. The DWHOS affected 2 different but connected ecosystems: the open ocean of the Gulf of Mexico and the nearshore

environments of the northcentral Gulf of Mexico (Peterson et al. 2012). The nearly 2000 m deep-water source of oil in the open ocean portion of the oil spill represented a unique event for both oil spill response and assessment of impacts to deep-water reefs and deep-sea benthic, bathypelagic, mesopelagic, epipelagic and *Sargassum* communities (e.g. White et al. 2012, Montagna et al. 2013, Powers et al. 2013, Fisher et al. 2014). In contrast, nearshore environments are often the final repositories of oil advected to the water's surface. Negative impacts of oil spills on indi-

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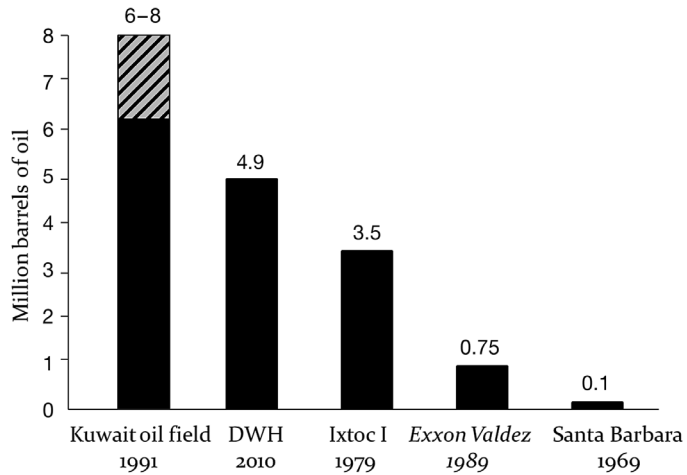


Fig. 1. Comparative oil volumes released during major oil spills throughout the world. Kuwait oil fields (Gulf War, Kuwait), *Deepwater Horizon* (DWH) (Northern Gulf of Mexico), Ixtoc I (Southern Gulf of Mexico), *Exxon Valdez* (Prince William Sound, Alaska) and Santa Barbara (California)

vidual species are well established in nearshore environments and include lethal as well as sublethal effects. Nearshore fauna is particularly susceptible to the detrimental effects of oil, which can result in reduced growth, recruitment failure, mortality and reduced fecundity (e.g. Burns & Teal 1979, Fleeger & Chandler 1983, DeLaune et al. 1984). Although prior studies allow for general predictions on the direction of species responses, the elucidation of injury pathways, quantification of injuries (Baker et al. 2017, this Theme Section) and scaling of restoration (Peterson & Lipcius 2003)—all of which are necessary components of the Natural Resource Damage Assessment (NRDA) process—represented a fundamental challenge to marine ecologists investigating the DWHOS.

### Nearshore studies of injury from the DWHOS

The very large temporal and spatial scale of the DWHOS prevented a comprehensive ecosystem study of injury to the full suite of natural resources potentially harmed. Even if one focused exclusively on nearshore ecosystems, ignoring the effects on offshore pelagic, mesopelagic and deep-water habitats, a comprehensive ecosystem study would need to encompass an area stretching from Texas to Florida (1000s of km of coastline) and sample across multiple years and multiple trophic levels at appropriate spatial and temporal scales. The lack of rigorously and/or systematically collected baseline samples (which were not possible since the scale of the spill

was not known until well into the event) prevented a Before-After-Control-Impact (BACI) study design (Underwood 1991). Furthermore, available baseline data were either extremely geographically restricted, or were collected as part of fisheries assessments that were never designed to have the statistical power to detect the effects of episodic events or determine the absolute density of animals. The absence of rigorous 'before' data limited most studies about the effects of the DWHOS to reference-impact designs.

The suite of targeted studies conducted by the DWHOS NRDA demonstrated significant ecological injury to the northern Gulf of Mexico. The results of studies presented in the present Theme Section as well as other studies on the DWHOS encompass the documentation of lethal and sublethal effects of oil spills on individual taxa (see Baker et al. 2017), spanning the full range of trophic levels from microbes (Dubinsky et al. 2013) to marine mammals (Barron 2012, Schwacke et al. 2014). Although many nearshore studies showed localized impacts of the DWHOS, not all nearshore studies indicated negative responses of key species (see Fodrie et al. 2014). Of those studies that did not detect any effects, many did not include areas of heavy oiling (such as Barataria Sound or Black Bay, Louisiana), were initiated >1 yr after oil washed ashore, focused on highly mobile taxa for which exposure may have been limited, or defined impacts as those that only showed population-level effects. Martin (2017, this Theme Section) demonstrated that behavioral avoidance of oil (non-weathered to medium-weathered) by some species may explain some failures to detect effects in those taxa.

### Recovery of nearshore ecosystems from the DWHOS

Anticipated recovery times of injured resources vary as a function of longevity of the species and impact on the habitats that the species rely upon (Baker et al. 2017, Zengel et al. 2017, this Theme Section). In general, habitat degradation or loss as a consequence of oiling or response-related activities will affect multiple generations of taxa and persist until restoration becomes effective. Several papers in this Theme Section highlight the complexity of effects that occur when habitat-forming species are injured by oiling or associated response activities. For example, Powers et al. (2017a) demonstrated how the loss of fringing oyster reef as a result of the oil spill can accelerate coastal erosion of marsh habitat.



Kenworthy et al. (2017) documented a loss of over 0.4 km<sup>2</sup> of seagrass meadows in the Chandelier Islands, Louisiana, as a result of the oil spill. Powers et al. (2017b) and Grabowski et al. (2017) demonstrated the loss of some 1.2 to 3 billion adult oysters from subtidal reefs in Louisiana. Studies conducted as part of the NRDA (Hester et al. 2016) as well as other investigations (e.g. Silliman et al. 2012) indicated substantial habitat degradation and loss resulting from accelerated erosion of coastal saltmarshes. Changes in the quantity and quality of essential habitats will directly affect many ecosystem services (e.g. nutrient regulation, fish habitat functions, shoreline stabilization, denitrification and carbon sequestration).

Studies published in this Theme Section and elsewhere expand upon the paradigms already established based on the *Exxon Valdez* oil spill on nearshore communities and show that: (1) organismal effects of heavy oiling occur across the full range of trophic levels; (2) oiling of foundational species or 'ecosystem engineers' can result in complex and long-term direct and indirect negative effects on nearshore ecosystems (Peterson et al. 2003); (3) ecological injury to biogenic habitats can result in loss of ecosystem services that cascade and affect other habitats and processes (Powers et al. 2017a); and (4) shoreline response activities intended to remove or mitigate the effects of oil are also associated with negative impacts on habitat-forming species (Driskell et al. 2001, Martínez et al. 2012, Peterson et al. 2012, Grabowski et al. 2017, Michel et al. 2017, this Theme Section, Powers et al. 2017a,b).

### Preparing for future oil spills

Assessments of ecological injury after future oil spills should develop and employ a conceptual model of injury pathways early in the assessment, and this model should focus heavily on habitat-forming species. Baker et al. (2017) provides the conceptual model adopted after the synthesis of results from the DWHOS. Such a conceptual model can also direct response action by prioritizing critical habitats in which oiling is likely to result in substantial habitat degradation or loss. In areas of intense oil and gas development or transport, resource managers should be proactive in adopting conceptual models of potential oil spills. Such models can strengthen the planning of response activities for future oil spills and direct researchers in establishing baseline data that can be used in future ecological assessments of oil spill effects.

The lack of baseline data for many of the natural resources affected by DWH oil and response activities prevented a full assessment of the long-term effects. Pennings et al. (2016) demonstrated how baseline data on marsh periwinkles can be used to support a conclusion of large-scale recruitment failure following the DWHOS. Similar recruitment failure likely resulting from the DWHOS occurred for oyster populations in the area (Grabowski et al. 2017), but the paucity of baseline data limited the power of such analyses.

Finally, any conceptual model should also include the potential effects from clean-up and response actions. Balancing the need to remove contaminants from the environment and the environmental damage and ecosystem costs associated with such response actions will always be a challenge for resource managers. Adoption of realistic conceptual models before the spill could allow the simulation of effects arising from the response actions. Minimizing long-term impacts to key habitat-forming species should be a goal of any assessment.

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# Nearshore exposure to *Deepwater Horizon* oil

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**ABSTRACT:** The *Deepwater Horizon* (DWH) oil spill affected more than 2000 km of shoreline. DWH oil entered the nearshore environment, stranding on shorelines as tar balls and/or emulsified oil, or forming submerged oil mats and integrating into nearshore sediments. The available chemistry data showed submerged sediments, especially within the first 50 m from oiled shorelines, displayed patchy distributions of elevated polycyclic aromatic hydrocarbon (PAH) concentrations in excess of ambient concentrations, which were quantified based on forensic findings establishing their source as being from the Macondo oil. Consistent with observed shoreline oiling conditions, PAH concentrations in the soils of affected Louisiana coastal wetlands were orders of magnitude higher than ambient concentrations, especially in locations along the seaward edge of the marsh. Both total and petrogenic PAHs decreased with distance from the shore in both inland and offshore directions. Although PAHs exhibited evidence of weathering over time, in the most heavily oiled areas, they continued to exceed ambient concentrations by orders of magnitude through fall of 2013.

**KEY WORDS:** *Deepwater Horizon* oil spill · National resources damage assessment · NRDA · Salt marsh · Nearshore · Submerged oil · PAH · Louisiana

## INTRODUCTION

On April 20 2010, an explosion aboard the *Deepwater Horizon* (DWH), a mobile, offshore Macondo prospect (Mississippi Canyon 252 or MC252) oil drilling rig in the northern Gulf of Mexico (GOM), caused the largest and most prolonged offshore oil spill in United States history. Response activities included application of large quantities of dispersants both at the wellhead and to offshore waters. As the oil traveled upward from the wellhead through the 1.5 km water column, it formed expansive surface oil slicks. Some of this oil was transported to nearshore communities by wind, currents, and water turbulence. Satellite imagery and other remote-sensing in-

formation demonstrated that the floating oil entered nearshore and estuarine areas of the northern Gulf of Mexico beginning in May 2010. Boufadel et al. (2014) estimated that 10 000 to 30 000 tons out of more than 440 000 tons of oil mass released reached the shoreline of GOM, affecting more than 2000 km of beach and marsh shorelines, of which more than 700 km were moderately to heavily oiled (Nixon et al. 2016).

DWH oil in the nearshore environment consisted of 4 distinct components: (1) floating oil slicks, sheens, and emulsions; (2) stranded oil in the form of tar balls and/or emulsified oil, which reached the land via tidal fluctuations, wind, and storm events; (3) submerged oil, which either sank when encountering suspended sediment in surface water or was trans-

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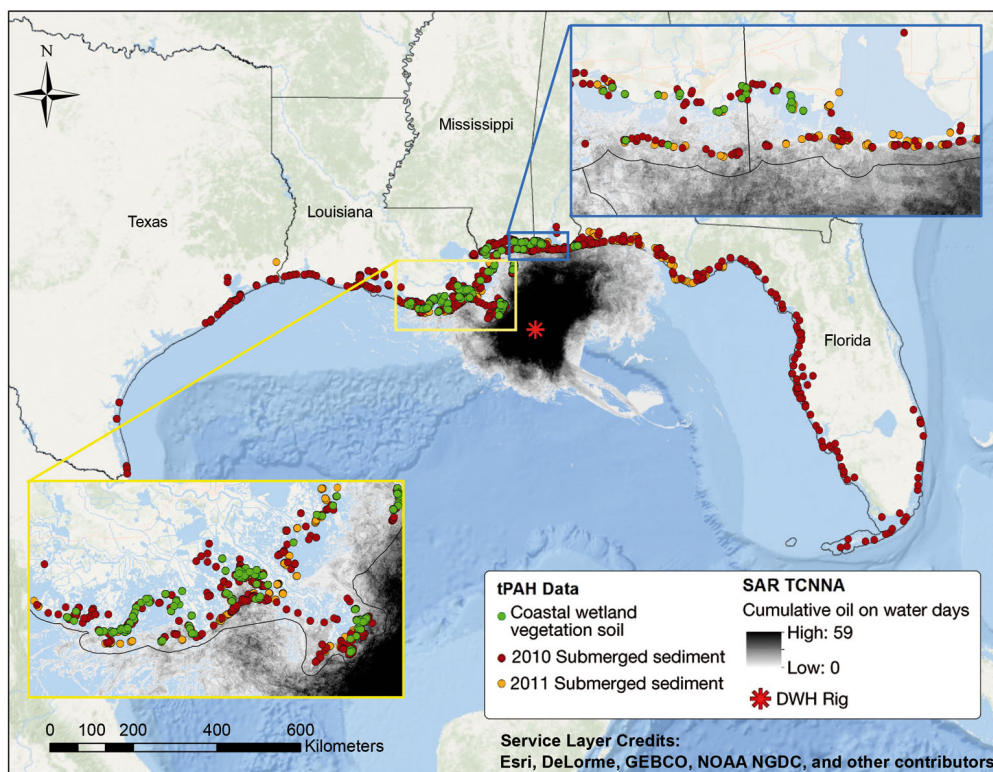


Fig. 1. Spatial distribution of oil on water and the nearshore chemistry data records in the Gulf of Mexico following the *Deepwater Horizon* (DWH) oil spill. Total polycyclic aromatic hydrocarbon (tPAH) data include coastal wetland vegetation soil samples (sampled from 2010 to 2013) and nearshore submerged sediment samples (sampled in 2010 and 2011). Daily extents of oil on water were delineated using SAR (synthetic aperture radar) images processed using the texture-classifying neural network algorithm (TCNNA)

ported via hydrodynamic wave action as it encountered surf resulting in flocculants, submerged oil mats (SOMs), and occasionally buoyant layers that appeared to be emulsified/oxidized oil and detritus (I. Zelo pers. comm.); and (4) dissolved oil present in nearshore water (Driskell & Payne 2015). The 4 components interact with each other. For example, stranded oil on shorelines can be re-mobilized to become submerged oil. The interactions among these components determined the fate of DWH oil within the nearshore environment.

The purposes of this study were: (1) to evaluate the spatial and temporal characteristics of nearshore submerged DWH oil; (2) to summarize the available polycyclic aromatic hydrocarbon (PAH) concentration data collected within coastal wetland environments in the northern GOM; and (3) to compare DWH oil exposures in various nearshore environment components and develop a conceptual model of nearshore exposure to DWH oil. Consistent with approaches used in past oil spills, PAH concentrations were used in order to indicate oil exposure and presence, determine the extent, magnitude and source of

contamination, and evaluate the recovery of exposed resources over time (O'Clair et al. 1996, Readman et al. 1996, Short et al. 1996, Carls et al. 2001, Brandt et al. 2002, Wang & Christensen 2006).

## MATERIALS AND METHODS

In order to quantitatively evaluate the exposure of the nearshore environment to DWH oil, more than 30 000 oil, soil, sediment, and tissue samples were collected. The present study focuses on US coastal wetland vegetation (CWV) soil data (1894 samples), which were collected between 2010 and 2013, and nearshore submerged sediment data (1731 samples), which were collected in 2010 and 2011, as depicted in Fig. 1.

### CWV soil sampling design

As part of the natural resources damage assessment (NRDA), shoreline and plant oiling were recorded between May and September 2010 at 2779



locations along the coastline of the northern GOM from White Lake in western Louisiana through Saint Marks in Florida (NOAA 2010), referred to as pre-assessment (PA) surveys. These surveys were intended to provide a global assessment of vegetation oiling conditions along the shoreline, although some locations experienced oiling after surveys were completed. The compiled information included observations of plant species, height of dominant vegetation, sediment oiling, and maximum length of oiled portions of plant stems. Plant stem oiling was computed as the ratio of oiled portion of plant stems to dominant vegetation mean height, and was used in the development of the subsequent CWV sampling plan (NOAA 2011a). The CWV sites in Louisiana were selected from PA sites using a stratified random sampling plan. Specifically, the PA sites along Louisiana vegetated shorelines were divided into 20 strata according to 5 classes of plant stem oiling and 4 habitat types. Plant stem oiling classes observed during PA surveys (hereafter 'PA plant oiling classes') were: (1) 0%, which served as the reference condition; (2) 0–10% (or trace to 10%); (3) >10 to 50%; (4) >50 to

90%; and (5) >90 to 100%. The 4 Louisiana habitat types were: (1) mainland herbaceous salt marsh; (2) back barrier herbaceous salt marsh; (3) coastal mangrove marsh; and (4) delta *Phragmites* marsh. Within each stratum, a given number of PA sites were randomly selected for CWV surveys to attain at least 95% confidence ( $\alpha = 5\%$ ) and 80% power ( $\beta = 20\%$ ). Each Louisiana mainland herbaceous salt marsh stratum contained the same number of selected sites. Such a balanced plan could not be achieved in other Louisiana habitats due to limited numbers of PA sites in various oiling classes. In Mississippi and Alabama, all the oiled PA sites were selected, with an equal number of reference sites included for CWV surveys (Willis et al. 2016). In total, 188 CWV sites were selected and repeatedly surveyed across the GOM over multiple seasons. The areal extent is depicted in Fig. 2 and the dates of surveys are listed in Table 1.

A transect was established at each CWV site. The initial transect length for the oiled Louisiana herbaceous marsh sites (mainland and back barrier) was set as the maximum visually observed extent of oil penetration as determined by either the PA survey or

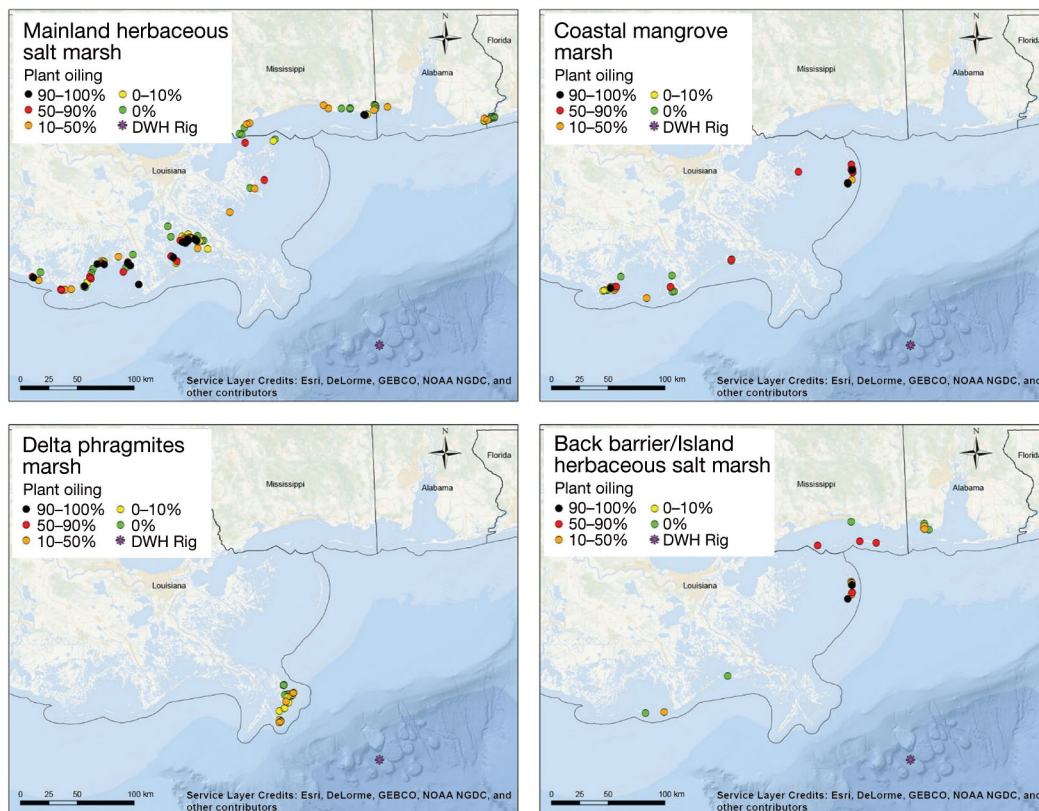


Fig. 2. Distribution of coastal USA wetland vegetation (CWV) sampling sites following the *Deepwater Horizon* (DWH) oil spill by habitat type and pre-assessment (PA) plant oiling class. Plant stem oiling was computed as the ratio of oiled portion of plant stems to dominant vegetation mean height

Table 1. Details of coastal wetland vegetation surveys carried out following the *Deepwater Horizon* (DWH) oil spill in April 2010. Dates are shown as mm/dd/yyyy

Survey season	Start	Finish	Extent of survey	Investigated sites
Fall 2010	9/16/2010	1/7/2011	All Louisiana sites	150
Spring 2011	4/9/2011	6/8/2011	All Louisiana, Mississippi and Alabama sites	188
Fall 2011	9/17/2011	11/2/2011	All Louisiana, Mississippi and Alabama sites	188
Fall 2012	9/10/2012	12/9/2012	All Louisiana, Mississippi and Alabama sites	188
Fall 2013	9/23/2013	12/13/2013	Louisiana (mainland + mangrove) sites, Mississippi and Alabama sites	152

CWV transect establishment team. The observed length of oil penetration into the vegetation varied from 2.9 to 30.0 m. The results indicate higher mean penetration lengths along heavily oiled sites with 90 to 100% plant oiling. The transect lengths for other CWV sites were set at 20 m or the maximum feasible length. Each transect had 1 to 3 fixed locations (Zone 1, Zone 2, and Zone 3) representing edge and interior exposure zones, with a minimum of a 1 m buffer maintained between zones. The center of the edge (or Zone 1) was located approximately 1.5 m inland from the shoreline during the initial setup, the center of Zone 2 was located inland of the shoreline at 50% of the transect length, and the center of Zone 3 was located inland of the shoreline at 80% of the transect length (NOAA 2011a).

Each zone contained observation ('cover') and sampling ('productivity') plots. Surface soil-scoop samples were collected from the 4 corners of the 0.25 m<sup>2</sup> portions of the productivity plots and placed in 4-ounce jars for PAH measurement. These samples were homogenized and composited at the laboratory prior to the PAH analysis. Grain size and total organic carbon (TOC) samples were measured by using two 7.2 cm outer-diameter core samples collected from each plot to a depth of 10 cm. These samples were analyzed separately in the laboratory. The reported grain size and TOC values were the arithmetic averages of individual reported values per zone. All the soil samples were kept cold (4°C or on ice) during storage prior to analysis.

### Submerged sediment sampling design

Submerged sediment data were collected in 2010 and 2011. The compiled 2010 submerged sediment samples were grouped as either pre- or post-spill, depending on their location and sampling date relative to the cumulative daily oil-on-water maps (Graettinger et al. 2015). Many of these samples were clustered around shorelines of concern and thus did

not provide widespread spatial coverage. The 2011 submerged sediment data were collected as part of the NRDA's marsh edge sandy shore (MESSh) survey. Data were collected from 180 marsh-edge and 151 beach sites, which were selected based on a number of stratified random sampling plans across Louisiana, Mississippi, Alabama, and Florida (NOAA 2011b,c). The marsh-edge sites were co-located with the CWV sites. The beach sites were primarily co-located with the 2010 summer PA sites and were stratified by state and shoreline oiling exposures. Between July and September 2011, 2 replicate transects, 100 m apart, were established at each site perpendicular to the shore on each side of the site center point. Transects were divided into 4 intervals extending to 0–10, 10–20, 20–50, and 50–500 m from the shore. At each transect, 10 cm or 7.6 cm outer diameter cores were collected at random within each of the 4 intervals across 2 depth horizons (0–2 and 2–4 cm). An electronic number generator was used to ensure the random selection of sampling locations within the intervals. Prior to PAH analyses, all collected sediment samples were subjected to laboratory total petroleum hydrocarbons (TPH) screening for presence of oil-related compounds (NOAA 2011b). Samples indicating likely presence of oil, along with their nearby unoiled samples, were subjected to PAH analysis. A minimum of 2 samples per site were selected randomly and analyzed for PAHs from any site without an oiled sediment sample.

### Chemistry data

The primary exposure data summarized in this work are total polycyclic aromatic hydrocarbon (tPAH) surrogate-corrected concentrations, calculated based on the summation of 50 PAH analytes including parent PAHs and selected alkylated homologs listed in Forth et al. (2015). If the concentration of a given compound in a sample was not detected, it was treated as a 0 value in the summation. PAHs, in-

cluding alkyl homologues, were analyzed in compliance with modified EPA method 8270D (EPA 2007), utilizing gas chromatography with low resolution mass spectrometry in the selected ion monitoring mode.

A quarter of the CWV soil samples and three-quarters of the nearshore submerged sediment samples evaluated in this work were also subjected to forensic analysis. Based on evaluation of dominant hydrocarbon types, quantitative comparison of diagnostic geochemical biomarker source ratios and spatial proximity to other indicators of DWH oil, the investigated samples were categorized into 5 match classification codes (Emsbo-Mattingly 2015a), i.e. A: consistent with fresh or weathered DWH oil; B: mostly consistent with fresh or weathered DWH oil; C: some weathered DWH oil may be present; D: indeterminate; or E: elevated presence of non-DWH oil. A description of the forensic analysis procedure is provided in the Supplement at [www.int-res.com/articles/suppl/m576p111\\_supp.pdf](http://www.int-res.com/articles/suppl/m576p111_supp.pdf) (see also Table S1 in the Supplement). Match code A and B (A/B) samples were further analyzed to quantify the depletion of DWH oil, which was computed as the percent change of the ratio of petrogenic PAHs to hopane in each sample relative to the ratio of the fresh DWH oil (Emsbo-Mattingly 2015a). All chemistry and forensic data were compiled in 2 web-based sources maintained by the National Oceanic and Atmospheric Administration (NOAA): Data Integration Visualization Exploration and Reporting (DIVER) (<https://dwhdiver.orr.noaa.gov/>); and Environmental Response Management Application (ERMA®) (<http://response.restoration.noaa.gov/erma/>).

#### **Statistical summarization of CWV soil chemistry data**

The tPAH CWV soil data were grouped by state/region, habitat, season, PA plant oiling class, and zone. Mississippi and Alabama sites along the Mississippi Sound were treated as members of one state/region group. Reported statistics of each group included sample size, mean, standard error, minimum, and maximum. In addition, when sample sizes exceeded 4, p-values corresponding to significances of 2-sample tests between tPAH concentrations at oiled groups and their corresponding reference values were calculated. For this purpose, parametric *t*-test and non-parametric Mann-Whitney *U*-tests were used concurrently. The computed *t*-test significances and standard errors were weighted and corrected for

finite populations. A plant oiling group was considered as having significantly higher tPAH concentrations if its mean exceeded the corresponding reference value with a p-value  $\leq 0.05$ .

Additional summarizations were performed by considering shoreline oiling exposure categories as the domains of study (Cochran 1977). These oiling categories were established via repeated shoreline oiling observations made from May 5, 2010 to March 25, 2014 as part of the Shoreline Cleanup Assessment Technique (SCAT) and Shoreline Rapid Assessment (RA) surveys (Nixon et al. 2016). The SCAT teams systematically surveyed fixed, linear segments of shoreline post-spill to support decision-making for shoreline cleanup. RA surveys focused on linear along-shore zones across a subset of the potentially impacted CWV areas in Louisiana. Each shoreline segment was assigned to a specific oil exposure category based on the observed maximum precedent oiling conditions. Shoreline oiling exposure categories for vegetated shorelines consisted of (1) heavier persistent oiling (heavy or moderate oiling was observed repeatedly over a period of 12 wk or longer); (2) heavier oiling (moderate or heavy oiling persisted for less than 12 wk); (3) lighter oiling (only trace to light oiling was observed); (4) no oil observed; and (5) not surveyed.

For the purposes of shoreline oiling summarizations and to avoid biases given the stratified random CWV sampling design from finite PA site populations, each tPAH measurement was assigned a sampling weight in accordance to its state/region, habitat, and plant oiling stratum. The sampling weight was calculated as the number of PA sites in a stratum divided by its corresponding number of CWV sites (Cochran 1977). All computations were performed using R package survey (R version 3.2.0) and verified by SPSS Complex Samples (IBM SPSS version 23).

#### **Statistical summarization of submerged sediment chemistry data**

The 2011 MESSh survey resulted in 5182 submerged sediment samples. Although screening determined 4326 of these samples as unoiled, only 14% (613) of these unoiled samples were subjected to PAH analysis. Summary statistics of 2011 MESSh data were generated by assigning a sampling weight to each 2011 MESSh sample (Cochran 1977) to avoid biases due to stratified random sampling from finite populations. The sampling weight of marsh-edge samples was determined based on the weight of their



co-located CWV sites. The sampling weight of each beach sample was calculated as the ratio of the total number of summer 2010 PA sites to the selected number of 2011 MESSh sites in the given stratum. Furthermore, the sampling weight of each unoiled sample was adjusted by the ratio of total number of unoiled samples collected to the number of unoiled samples analyzed for PAHs in the given stratum. For such data, computed summary statistics included weighted mean, standard deviation, as well as minimum and maximum values. All other tPAH data investigated in this work, including 2010 post-spill, were collected in accordance to unstratified plans, and hence, were not subjected to any sampling weight. For such data, computed summary statistics included unweighted mean and standard deviation, as well as minimum and maximum values.

## RESULTS

### Ambient concentrations

Ambient concentrations are key components of the exposure analysis. In this work, determination of ambient concentrations is complicated by the vastness of the investigated area, containing regions and zones with substantially different characteristics. For example, the delta region is heavily influenced by the Mississippi River runoffs, while the remote barrier islands are not only less affected by shore-based contamination, but also possess more active hydrodynamic environments when compared to nearshore coastal areas. To incorporate such regional characteristics, an innovative procedure based on forensic chemistry was devised. For this purpose, forensic results were used to identify ambient representative CWV soil and submerged sediment samples along various states and habitats. Each ambient representative sample was selected based on 2 conditions: (1) the sample was forensically identified as code D, i.e. the sample does not match any field or DWH oil due to numerous non-detects or interferences (Emsbo-Mattingly 2015a), and (2) the sample was located at least 100 m from any DWH oil manifestation. These manifestations included shoreline segments or sites observed to be oiled by various survey teams, as well as tar ball, oil sheen, soil, sediment and tissue samples categorized as forensic code A or B. The purpose of the 100 m distance was to minimize the chances of having diluted DWH oil in ambient representative samples.

Summary statistics of ambient representative tPAH samples for various states, habitat types and dis-

tances from the shore are listed in Table 2. Among the computed ambient tPAH concentration ranges, the highest values were from the Louisiana Delta *Phragmites* marsh area. In contrast, the lowest ambient tPAH concentrations occurred along barrier islands. Different ambient tPAH concentrations may partially be attributed to regional variations of TOC and grain size. In Louisiana Delta *Phragmites* marsh, the mean CWV soil TOC was 2%, with an average of 60% fine particles. The mean CWV soil TOC in barrier islands was measured at 1%, with mean percent fine particles of 34%.

### Submerged sediment chemistry data

The compiled data from 2010 post-spill and 2011 MESSh were separated into subsets based on their nearest shoreline oiling exposure category and vegetation status, as defined by Nixon et al. (2016). While most non-vegetated subsets provided adequate sample sizes (in excess of 8 samples), the only vegetated subsets large enough to warrant further analysis were along Louisiana shorelines, as shown in Table 3. This is primarily due to the fact that oiled shorelines in the other investigated states were predominantly non-vegetated, whereas oiled shorelines in Louisiana were dominated by vegetation (Nixon et al. 2016).

Nearshore tPAH concentrations show a clear decreasing pattern as distance to shore increases, with the highest values concentrated within the first 50 m from the shore, as depicted in Fig. 3 for Louisiana mainland salt/brackish herbaceous marsh sites. In this figure, ambient concentration ranges are shown as shaded intervals. Similar ranges for different shorelines and oiling exposures during 2010 and 2011 are provided in Figs. S1–S4 in the Supplement at [www.int-res.com/articles/suppl/m576p111\\_supp.pdf](http://www.int-res.com/articles/suppl/m576p111_supp.pdf), respectively. In all these figures, each sample is color coded based on its forensic code, with non-detects displayed as 0.001 parts per billion (ppb) values. These figures consistently indicate that the forensic code A/B samples with highest tPAH concentrations were situated primarily within the first 50 m from the shore. Summary statistics of 2010 post-spill and 2011 MESSh data, grouped by state, shoreline habitat type and oiling categories, and distance from the shore, are shown in Tables S2 & S3 in the Supplement, respectively.

Comparison of the ambient tPAH concentration ranges in Table 2 with 2010 post-spill and 2011 MESSh results, indicates presence of elevated concentrations along oiled shorelines, especially within the

Table 2. Summary statistics of ambient representative total polycyclic aromatic hydrocarbon (tPAH) concentrations following the DWH horizon oil spill in April 2010 through fall of 2011. Samples were taken from coastal wetland vegetation (CWV) soil, in submerged sediment within 50 m from the shore (Sediment 0–50) and in submerged sediment between 50 and 500 m from the shore (Sediment 50–500)

State	Habitat	Location	Sample size	tPAH concentrations (ppb)			
				Mean	SD	Min	Max
Louisiana	Mainland herbaceous salt marsh	CWV soil	24	278	169	51	737
		Sediment 0–50	58	264	422	8	2934
		Sediment 50–500	106	167	125	9	828
	Back barrier herbaceous salt marsh	CWV soil	6	26	20	2	46
		Sediment 50–500	5	41	43	7	105
	Coastal mangrove marsh	CWV soil	20	244	238	0	766
		Sediment 0–50	3	74	1	73	75
		Sediment 50–500	6	109	109	7	238
	Delta <i>Phragmites</i>	CWV soil	18	4278	5918	1211	24448
		Sediment 0–50	59	3015	3049	206	13521
		Sediment 50–500	57	1818	1920	425	13130
	Non-vegetated	Sediment 0–50	4	718	707	105	1506
		Sediment 50–500	43	513	664	0	2067
Mississippi/Alabama (Mississippi Sound)	Mainland herbaceous salt marsh	CWV soil	30	254	225	19	953
	Island herbaceous salt marsh	CWV soil	12	130	108	7	358
Mississippi	Non-vegetated	Sediment 0–50	11	1755	3313	3	9780
		Sediment 50–500	26	67	189	0	772
Alabama	Non-vegetated	Sediment 0–50	8	124	218	0	640
		Sediment 50–500	38	68	130	0	526
Alabama (Perdido Bay) Florida	Mainland herbaceous salt marsh	CWV soil	9	210	278	3	679
		Sediment 0–50	45	100	201	0	896
		Sediment 50–500	58	152	412	0	2084

first 50 m from the shore. The spatial presence of DWH oil is further confirmed by forensic code A/B samples, as shown in Fig. 4. This figure also shows the forensic code A/B classifications among stationary and chain-drag sentinel samples. These sentinel results confirm the presence and/or passage of oil in the water column and oil at the sediment–water interface.

### CWV soil chemistry data

CWV soil chemistry data are available from fall 2010 to fall 2013 at a maximum of 188 fixed sites along the GOM. These measurements were taken from various habitats in Louisiana, Mississippi, and Alabama, which were investigated systematically

Table 3. Counts of submerged nearshore sediment samples with tPAH results in 2010 and 2011, by state, shoreline oiling category and shoreline type

Shoreline oiling category	Shoreline type	Sample size (2010/2011)				
		Louisiana	Mississippi	Alabama	Florida	Texas
(1) Heavier persistent oiling	Vegetated	64/161	0/0	0/0	0/0	0/0
	Non-vegetated	15/17	1/7	0/0	0/0	0/0
(2) Heavier oiling	Vegetated	139/180	0/0	0/0	0/0	0/0
	Non-vegetated	38/65	8/14	35/85	33/36	0/0
(3) Lighter oiling	Vegetated	98/229	1/17	3/2	0/0	0/0
	Non-vegetated	42/68	45/39	29/38	40/34	17/0
(4) No oil observed	Vegetated	154/420	12/14	11/54	6/5	0/0
	Non-vegetated	41/10	7/28	8/30	35/75	0/0
(5) Not surveyed	Vegetated	72/167	7/3	9/7	88/78	14/0
	Non-vegetated	4/0	0/0	0/0	62/8	37/0
Total		667/1317	81/122	95/216	264/236	68/0

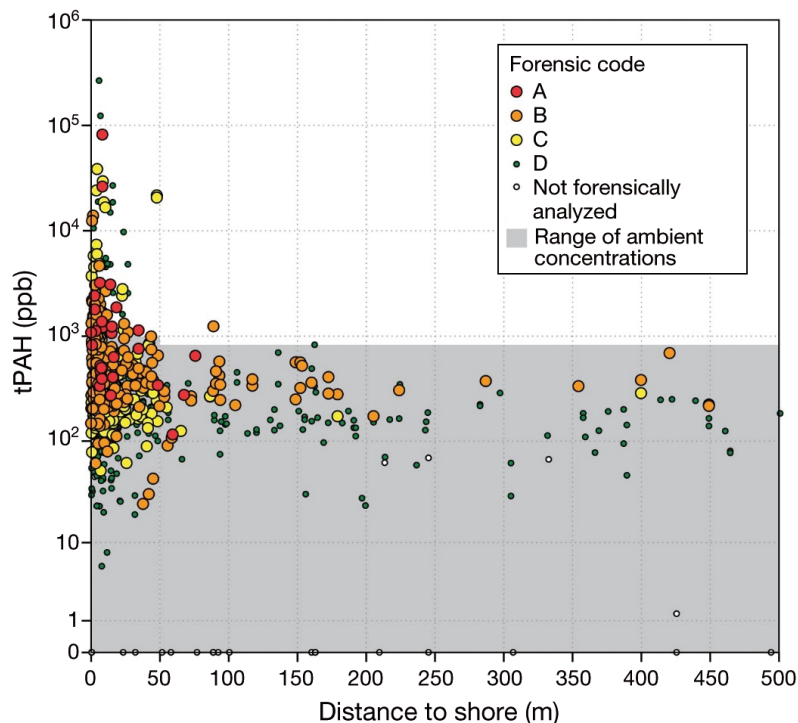


Fig. 3. Nearshore submerged sediment tPAH concentrations versus distance to shore along Louisiana mainland herbaceous salt marsh shorelines following the DWH oil spill. Samples were collected in 2010 and 2011. Forensic codes: A = consistent with fresh or weathered DWH oil; B = mostly consistent with fresh or weathered DWH oil; C = some weathered DWH oil may be present; D = indeterminate

beginning in the fall of 2010 in Louisiana and spring of 2011 in Mississippi and Alabama through the fall of 2013. This dataset was used to quantitatively evaluate the spatial and temporal patterns of DWH oil exposure in various coastal wetland soils.

Forensic results confirmed the ubiquitous presence of DWH oil within impacted coastal wetlands (Fig. 5), especially those within Louisiana mainland herbaceous salt marshes. Along Louisiana mainland herbaceous salt marshes, 77% of samples from sites with 90 to 100% plant oiling, and 99% of samples from sites adjacent to heavier persistent oiling shorelines, were identified as either forensic code A or B (Table 4). The complete set of forensic results, classified by plant oiling classes and shoreline oiling exposure categories in various states and habitats, are summarized in Tables S4 & S5 in the Supplement, respectively.

As noted, consistent with the stratified random CWV sampling plan, tPAH data were grouped by state/region, habitat type, season, PA plant oiling class, and zone. Weighted summary statistics for each group, including sample size, mean, standard error, minimum, maximum, and p-values, are presented in Table S6 in the Supplement. Plant oiling groups with significantly elevated tPAH concentrations relative to their corresponding reference values are highlighted as those with p-values less than or equal to 0.05. Significantly elevated tPAH concentrations were mainly found along Louisiana mainland herbaceous salt marshes and occasionally back barrier herbaceous salt marshes and mangroves. The highest concentrations were generally reported along the oiled shoreline edge or Zone 1. For example, in the fall of 2010, at Louisiana mainland herbaceous salt marsh sites with 90 to 100% plant oiling, the mean tPAH concentration in Zone 1 was 65 300 ppb, while Zones 2 and 3 (the interior exposure zones) reported 7420 and 6050 ppb, respectively (as shown in Table S6).

Although many oiled sites had tPAH concentrations that were significantly higher than

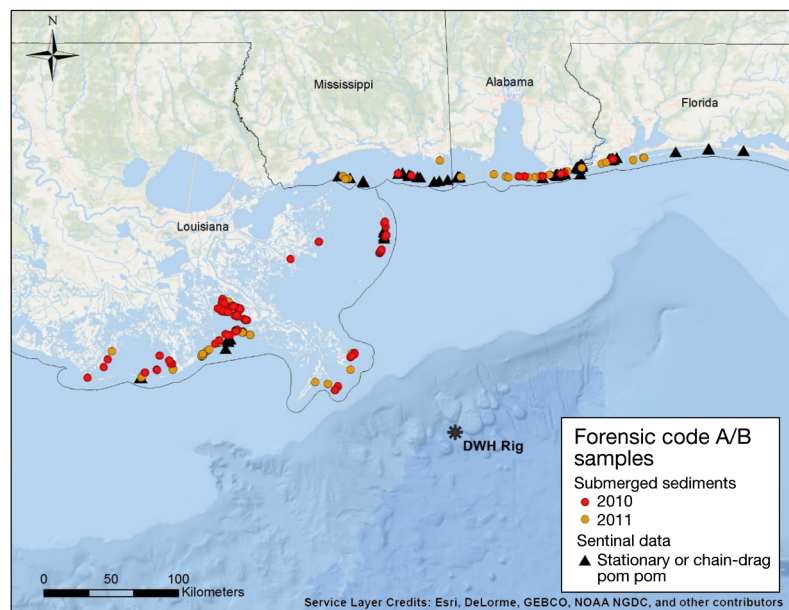


Fig. 4. Spatial distribution of forensic code A/B samples for submerged sediments and sentinel data along the northern coast of the Gulf of Mexico following the DWH oil spill. Forensic codes A/B are consistent (A) or mostly consistent (B) with fresh or weathered DWH oil

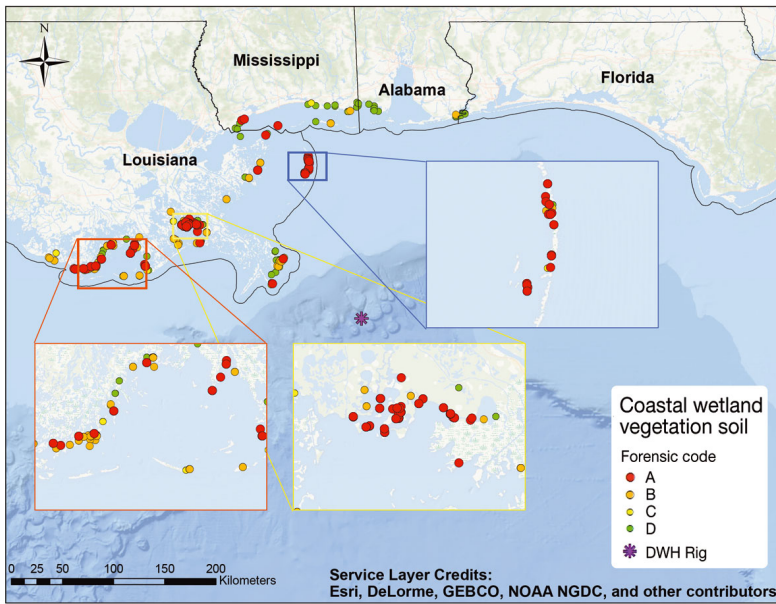


Fig. 5. Spatial distribution of coastal wetland vegetation (CWV) soil along the northern coast of the Gulf of Mexico following the DWH oil spill by results of forensic analysis. For key to forensic codes (A to D) see Fig. 3 legend. CWV soil samples collected in fall 2010 and spring 2011 were forensically analyzed; samples collected afterwards were not forensically analyzed. The three insets provide additional detail for areas where many sites are clustered and individual forensic codes may not be clear

reference values, these concentrations did not display consistent, increasing trends relative to plant oiling. For example, mean tPAH concentrations at Louisiana mainland herbaceous salt marsh sites in Zone 1 in fall 2010 were 974 ppb at reference sites (0% plant oiling) and 3350, 55 100, 14 100 and 65 300 ppb at sites with 0–10, 10–50, 50–90 and 90–100% plant oiling, respectively.

The absence of a consistent, increasing trend between tPAH concentrations and PA plant oiling may be attributed to delayed oiling at some of the CWV sites; this includes sites that were heavily oiled after the summer 2010 PA survey but before the CWV surveys. In order to address delayed oiling, summary statistics based on maximum precedent shoreline oiling exposure categories were computed (Table S7 in the Supplement). For this purpose, exposure categories were treated as domains of study. Weighted statistics by states, habitat types, seasons, shoreline oiling exposure categories, and zones included sample size, mean, standard error, minimum, maximum, and p-values. The listed p-values are associated with *t*-tests and

Mann-Whitney *U*-tests between tPAH concentrations in oiled shoreline groups and their corresponding no-oil-observed values. An oiled shoreline group is considered as having significantly elevated tPAH concentrations if its mean exceeds the corresponding mean no-oil-observed value with a *p*-value  $\leq 0.05$ . Significantly elevated tPAH concentrations occurred mainly along oiled shorelines of Louisiana mainland herbaceous salt marshes and occasionally in back barrier herbaceous salt marshes and mangroves, where the highest concentrations were reported along their edges. CWV soil data from other states and habitats were insufficient to determine significant tPAH occurrences above their corresponding no-oil-observed values.

When maximum precedent shoreline oiling exposure categories were used, the mean CWV soil tPAH concentrations displayed consistent, increasing patterns relative to shoreline oiling exposure categories. For example,

the mean tPAH concentrations at Louisiana mainland herbaceous salt marsh sites in Zone 1 in fall 2010 were 394 ppb at sites with no oil observed and 4480, 12 400 and 128 000 ppb at sites with lighter, heavier, and heavier persistent oiling, respectively.

Table 4. Counts (percentages) of CWV soil samples within each forensic code in Louisiana mainland herbaceous salt marsh following the DWH oil spill, grouped by pre-assessment (PA) plant oiling class and shoreline oiling category. Forensic codes: A = consistent with fresh or weathered DWH oil; B = mostly consistent with fresh or weathered DWH oil; C = some weathered DWH oil may be present; D = indeterminate. Plant stem oiling was computed as the ratio of oiled portion of plant stems to dominant vegetation mean height

	Forensic code			
	A	B	C	D
<b>PA plant oiling class</b>				
90–100%	35 (37)	38 (40)	10 (11)	12 (13)
50–90%	41 (43)	28 (29)	8 (8)	18 (19)
10–50%	58 (50)	35 (30)	9 (8)	14 (12)
0–10%	14 (19)	21 (29)	8 (11)	29 (40)
0–0%	1 (1)	26 (24)	20 (18)	63 (57)
<b>Shoreline oiling exposure category</b>				
Heavier persistent oiling	86 (82)	18 (17)	1 (1)	
Heavier oiling	34 (32)	41 (39)	13 (12)	18 (17)
Lighter oiling	22 (17)	37 (28)	21 (16)	52 (39)
No oil observed	3 (3)	36 (37)	13 (13)	46 (47)
Not surveyed	4 (9)	16 (34)	7 (15)	20 (43)



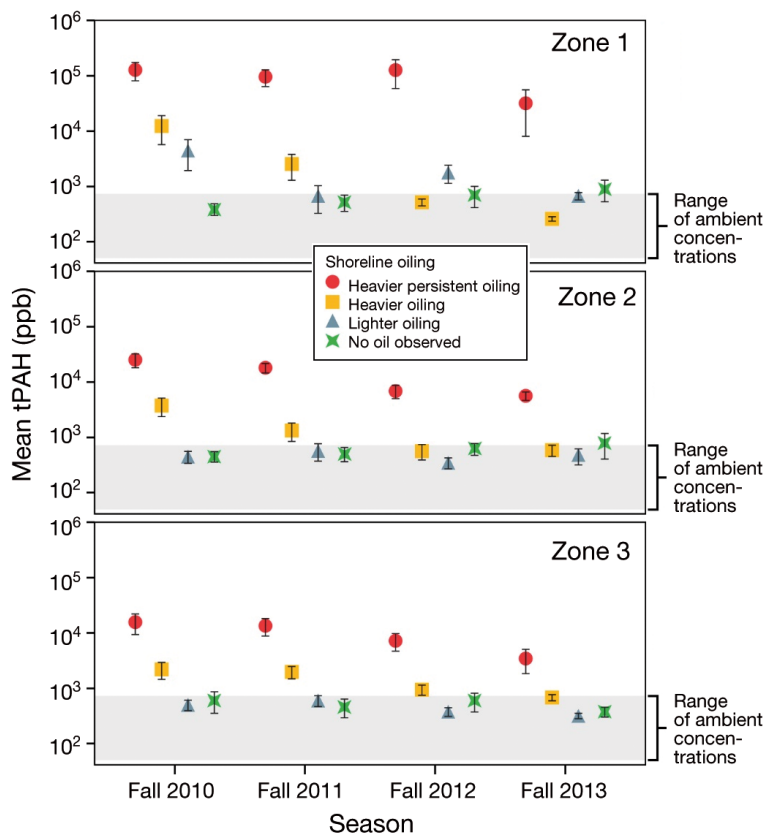


Fig. 6. Time series plots of soil tPAH concentrations for shoreline with different degrees of oiling in Louisiana mainland herbaceous salt marsh following the DWH oil spill in April 2010. Shaded areas show the range of ambient concentrations. Error bars represent  $\pm$  SE

Fig. 6 displays zone-specific time series plots of weighted mean tPAH concentrations along various shoreline oiling categories of Louisiana mainland herbaceous salt marshes. In this figure, ambient concentration ranges are shown as shaded intervals. The figure depicts the gradual decline of CWV soil tPAH concentrations along oiled shorelines since the fall of 2010. However, the tPAH concentrations along heavier persistent oiling shorelines in all zones remained elevated above ambient ranges through the fall of 2013. Graphs for other states and habitats are provided as Figs. S5–S10 in the Supplement.

In the above analyses, fully eroded plots were considered as missing values. As indicated by the decreasing CWV sample sizes between the fall of 2010 and 2013 (Tables S6 & S7), surveyed sites experienced various levels of land losses. For example, the number of Zone 1 plots in Louisiana mainland herbaceous salt marsh sites adjacent to heavier persistent oiling shorelines decreased from 13 in the fall of 2010 to only 4 in the fall of 2013. Such drops in sample sizes reduced the power to distinguish differences between oil and reference sites.

## Weathering of DWH oil

Chemical weathering of DWH oil in the nearshore environment was investigated by focusing on petrogenic PAHs, i.e. 27 alkylated hydrocarbon compounds associated with petroleum, as listed in Emsbo-Mattingly (2015a). The depletion was calculated as the average percent difference between relative abundances of individual petrogenic PAHs (PetPAHs) in forensic code A/B soil and sediment samples and those measured in fresh Macondo oil collected from the wellhead. In this case, the relative abundance of a PetPAH is calculated as the ratio of its abundance to hopane, a conservative internal marker within the oil (Emsbo-Mattingly 2015a). The computed depletion rates are greatly influenced by the environmental conditions in which the oil resides. These rates are representative of depletion at the time of sampling, and are not time-normalized. Time-normalization was not possible due to the unknown time of the original release of the sampled oil.

Only data along Louisiana mainland herbaceous salt marshes were adequate for statistical summarizations, as listed in Table 5. These results indicate significant weathering of the DWH oil that penetrated vegetated shorelines. In both inland and offshore directions in 2010, the petrogenic PAH depletion rates were greater than 95% close to the shore and over 97% at further distances. Despite these already elevated rates, the petrogenic PAHs continued to deplete each year (Table 5).

## DISCUSSION

### Spatial and temporal patterns of oil stranded in CWV soil

The extensive CWV tPAH dataset was analyzed as a quantitative basis for evaluating temporal and spatial patterns of DWH oil exposures specifically along affected vegetated shorelines of Louisiana, Mississippi, and Alabama. As demonstrated by the forensic results, samples from vast regions of the northern GOM displayed chemical signatures of DWH oil. These samples included those collected at locations that had been previously identified as either no-oil-

Table 5. Summary statistics of petrogenic polycyclic aromatic hydrocarbon (PAH) depletion rates along Louisiana mainland herbaceous salt marsh shorelines following the DWH oil spill based on forensic code A/B samples. Forensic codes A/B are consistent (A) or mostly consistent (B) with fresh or weathered DWH oil. Data are shown for coastal wetland vegetation (CWV) soils in 3 zones, with Zone 1 representing water edge conditions (approximately 1.5 m inland from the shoreline) and Zones 2 and 3 conditions progressively further inland; and for submerged sediments in 2 zones, 0–50 m and 50–500 m from the shore. These rates represent depletion at the time of sampling, and are not time-normalized

Matrix	Position	Sample size	Petrogenic PAHs depletion (%)			
			Mean	SD	Min	Max
<b>2010</b>						
CWV soil	Zone 3	35	97.5	1.0	94	99
	Zone 2	38	97.5	0.8	95	99
	Zone 1 (edge)	40	95.7	2.2	90	98
Submerged sediment	0–50 m from shore	90	95.8	2.2	87	99
	50–500 m from shore	11	97.5	1.1	95	99
<b>2011</b>						
CWV soil	Zone 3	36	97.5	1.8	91	99
	Zone 2	38	97.7	1.3	94	99
	Zone 1 (edge)	37	96.7	1.6	93	99
Submerged sediment	0–50 m from shore	103	97.2	1.6	87	99
	50–500 m from shore	25	97.4	0.6	96	98

observed by SCAT and RA teams, or reference sites (0% plant oiling) by CWV survey teams. For example, 40% of the samples collected at sites along no-oil-observed Louisiana mainland herbaceous shorelines were forensically identified as code A or B, albeit at concentrations lower than those measured at oiled sites. For the same habitat, 25% of the selected CWV reference sites were forensically identified as code A or B samples (Table 4). These results suggest that tPAH concentrations collected at locations identified as either reference or no-oil-observed cannot be automatically considered as representative of ambient conditions. Our forensic-based approach for determination of ambient concentrations addresses this conundrum and provides reliable, region-specific results.

The computed ambient concentrations are displayed in Fig. 6, which shows zone-specific time series plots of weighted mean tPAH concentrations along various shoreline oiling categories of Louisiana mainland herbaceous salt marshes. This figure depicts the gradual decline of CWV soil tPAH concentrations along oiled shorelines since the fall of 2010. Similar results were reported by Turner et al. (2014a,b) who investigated changes in the PAH concentrations subsequent to the DWH oil spill based on samples from a limited number of Louisiana marsh sites. Some of the tPAH concentration declines can be

attributed to the erosion of plots along the oiled marsh edges of Louisiana.

### Spatial and temporal patterns of nearshore submerged oil

The sediments affected by submerged oil were often overlain by a thin layer of flocculants containing oil. As described in Emsbo-Mattingly (2015b), sampling techniques implemented prior to 2011 were causing the loss of flocculants, likely resulting in underestimation of PAHs associated with sunken oil. Improved techniques resulted in the more efficient capture of flocculants during 2011 MESSh submerged sediment samplings.

As shown by our results, submerged sediments displayed patchy distributions of elevated PAH concentrations along oiled shorelines, with means 2 to 3 times higher than their corresponding ambient values, particularly ad-

acent to vegetated shorelines. PAH concentrations in submerged sediment were noticeably elevated above ambient values within the first 50 m from oiled shorelines. This 50 m range also contained the majority of forensic code A/B samples. Beyond the first 50 m from the shore, lower tPAH concentrations were observed with sporadic forensic code A/B samples.

Determination of the temporal patterns of submerged sediment tPAH concentrations is often facilitated when co-located samples measured at different times are available. Although 2011 MESSh provided a comprehensive and balanced coverage of impacted shorelines, 2010 submerged sediment data were clustered along shorelines of concern. In addition, 2010 reported concentrations were likely biased low due to sampling techniques prone to flocculent losses. Of the thousands of submerged sediment samples collected in 2010 and 2011, only 112 pairs were within 100 m of each other between the 2 years. Given the spatial extent of the impacted shorelines, such a sample size is very small. However, review of the results suggested trends associated with the temporal patterns of submerged sediment tPAH concentrations. Of the 2010/2011 paired samples, 64 (or 57%) indicated a decline in tPAH concentrations between 2010 and 2011. Approximately 36% (23) of these 64 samples were forensic code A/B in 2010. Of these 23 forensic code A/B samples, 83% (19) indicated a de-

cline in tPAH concentrations between 2010 and 2011. The mean tPAH concentration of the 23 forensic code A/B samples was 1014 ppb with standard error of 211 ppb, which decreased in 2011 to  $406 \pm 85$  ppb. This decrease was statistically significant with a parametric *p*-value of 0.013 (paired *t*-test) and a non-parametric *p*-value of 0.001 (Wilcoxon test).

Besides submerged oil tPAH data, SOMs, formed adjacent to beaches, were also observed during various response surveys (Michel et al. 2013). In general, SOMs are difficult to locate and require considerable skill to remove. According to Hayworth et al. (2011), SOMs can contain up to 90 percent sediment and range in thickness from a few millimeters to several centimeters. Oil in the interior of SOMs may resist weathering for many years, providing a source of oil and contaminants over time (Hayworth et al. 2011, Driskell & Payne 2015, Emsbo-Mattingly & Martin 2015).

### Previous oil spill exposures

Multiple authors (Lee & Page 1997, Nixon et al. 2013, Samaras et al. 2014) have explored the spatial and temporal distribution of nearshore exposure to past oil spills. These authors attributed the distribution of oil and its presence in the nearshore environment to a variety of factors, including oil type, coastal topography, beach permeability and stability, concentration of suspended sediments, and site-specific wave exposure. In studying the effects of the Exxon Valdez oil spill, Short et al. (2004) found that the most heavily oiled segments occurred within sheltered embayments where the largest initial landfall occurred. O'Clair et al. (1996) found some oil in deep subtidal sediments; however, the greatest concentration of oil was in shallow, nearshore submerged sediments at or near the land–water interface. Even at heavily oiled locations, there was little evidence of sediment contamination in water depths greater than 40 m. Similar results were also reported by Lee & Page (1997) based on a study of several spills. Studies of past spills, including the Exxon Valdez and Gulf War oil spills, also demonstrated that impacted soil and sediment samples adjacent to or within intertidal zones displayed elevated PAH concentrations consistent with observed oiling (O'Clair et al. 1996, Readman et al. 1996, Carls et al. 2001). These authors also demonstrated the gradual recovery of impacted resources, which was always accompanied by declining PAH concentration, although along the most heavily oiled locations, elevated PAH concentrations persisted years after the original impact.

### Conceptual model of nearshore exposure to DWH oil

In the case of the DWH oil spill, the spatial and temporal distribution of the nearshore exposure has been analyzed by relying on data collected in NRDA efforts. Stout (2015a) discussed the chemical characteristics of the floating oil (or oil-on-water), while the daily spatial extent of oil-on-water was determined based on information from 4 different satellite-based sensors (Graettinger et al. 2015). Driskell & Payne (2015) and Allan et al. (2012) explored the PAH patterns of the oil in water. PAH and forensic properties of stranded oil were studied by Stout (2015b) and Emsbo-Mattingly & Martin (2015).

Graettinger et al. (2015) presented the extent of oil-on-water for 68 d in 2010 subsequent to the DWH spill. Emsbo-Mattingly & Martin (2015) demonstrated that the oil-on-water experienced substantial weathering, with petrogenic PAHs depleting at 61% on mean (65% median) before reaching the nearshore. Allan et al. (2012) observed temporary, significant increases in dissolved bioavailable PAH concentrations as the floating oil reached the shoreline at a few specific locations. Driskell & Payne (2015) investigated a large number of 2010 nearshore water samples, including many from the first 50 m from the shore. They noted the general scarcity of particulate oil and the dominance of mostly dissolved- or indeterminate-phase oiled water samples. Of the investigated water samples, 36% were considered matches to DWH oil. The oil in these matching samples was predominantly attributed to leaching from previously deposited DWH oil, while the few samples with particulate components were related to re-suspended oiled sediments (Driskell & Payne 2015). Regarding stranded oil, Stout (2015b) and Emsbo-Mattingly & Martin (2015) conducted comprehensive forensic analyses of thousands of oil, tar ball, sheen, and soil samples. These results indicated widespread exposure to stranded DWH oil in the nearshore environments of the northern GOM.

To bring all these components together, a representative pictorial model of nearshore exposure was developed based on the results associated with forensic code A/B samples when compared with ambient representative samples. These results included 2011 CWV and 2011 MESSh samples along Louisiana mainland herbaceous salt marshes, whose summary statistics are displayed in Fig. 7. This figure indicates that within the stranded oil component of the model, the highest tPAH concentrations occurred along the seaward edge of marshes. These concentrations were



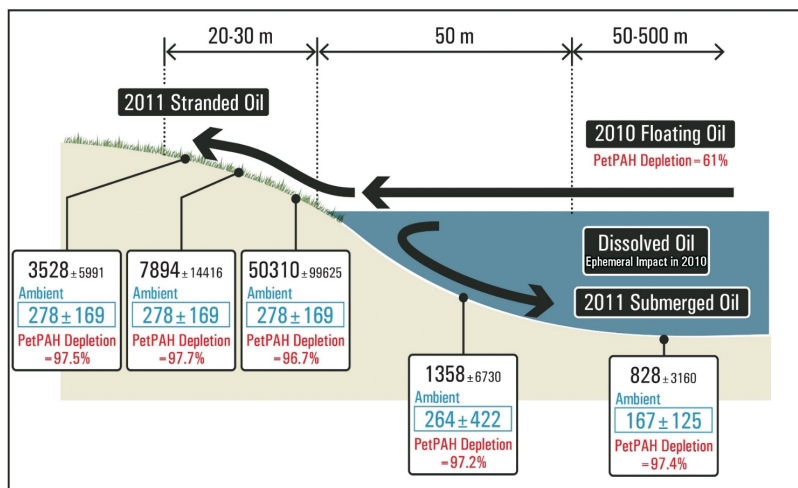


Fig. 7. Conceptual model of nearshore exposure to DWH oil based on 2011 forensic code A/B samples (for an explanation see Fig. 3) when compared to ambient samples along Louisiana herbaceous salt marsh shorelines. tPAH concentrations in 2011 (ppb, average ± SD) are displayed in black; ambient tPAH concentrations are displayed in blue. Average petrogenic PAH depletion rates in percent are shown in red. These rates represent depletion at the time of sampling, and are not time-normalized

orders of magnitude higher than their ambient levels (Table 2). In the submerged oil component of the model, the highest tPAH concentrations in excess of ambient levels occurred within the first 50 m from the shore. The elevated standard deviations of tPAH in Fig. 7 highlight the patchy distribution of DWH oil through the nearshore environment.

Comparison of the 2010 and 2011 mean petrogenic PAHs depletion rate, listed in Table 5, indicates a gradual increase in the weathering of both stranded and submerged oil as distances to the shore, in both inland and offshore direction, increase (Fig. 7). Although tPAH concentrations were generally declining, elevated concentrations above ambient levels persisted along the most heavily oiled shorelines into 2013. In addition, DWH oil exposure may continue through leaching and resuspension of submerged and stranded oil.

The dissolved DWH oil component, in contrast to stranded and submerged oil, experienced ephemeral impacts (Allan et. al 2012). These authors noted that within 1 yr after the arrival of the floating oil, nearshore water PAH concentrations reverted back to pre-oiling levels. Similar results were reported by Driskell & Payne (2015), who indicated that the percentage of water samples matching DWH oil peaked during the summer and fall of 2010, with a far lower percentage of matches in the spring of 2011.

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**Supporting information.** This material is freely available in the Supplement at [www.int-res.com/articles/suppl/m576/p111\\_supp.pdf](http://www.int-res.com/articles/suppl/m576/p111_supp.pdf). It includes (1) summary statistics of forensic results in various states, habitats, shoreline oiling and plant oiling categories, (2) summary statistics of measured tPAH concentrations in wetland soils in various states, habitats, periods, shoreline oiling and plant oiling categories, (3) graphical presentation of mean tPAH concentrations in wetland soils versus survey time in various states, habitats, and shoreline oiling exposures, (4) summary statistics of measured tPAH concentrations in various periods, states, habitats, and (5) graphical presentation of measured tPAH concentrations in submerged sediments versus distance from the shore in various periods, states, habitats, and shoreline oiling exposures.

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Contribution to the Theme Section

'Response of nearshore ecosystems to the Deepwater Horizon oil spill'



# Avoidance of oil contaminated sediments by estuarine fishes

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**ABSTRACT:** The explosion of the *Deepwater Horizon* drilling platform and subsequent discharge of hydrocarbons into the Gulf of Mexico is the largest oil spill that has affected US waters. To date, studies of ecological effects on coastal fishes have produced complex results. While laboratory studies indicate that oil has widespread, pervasive impacts on fish physiology, field assessments often document few impacts to fish abundance and biomass following a spill. The dichotomy of such results suggests that negative individual-level effects do not translate to population/community-level consequences. One explanation for this discrepancy is that mobile organisms may avoid oil, which was very patchily distributed. Here, I present the results of experiments using 3 estuarine fishes (gulf killifish *Fundulus grandis*, sailfin molly *Poecilia latipinna*, and sheepshead minnow *Cyprinodon variegatus*) to determine whether behavioral avoidance occurs at a range of concentrations (0, 10, 20, and 40 ml oil l<sup>-1</sup> of sediment) and weathering (fresh or weathered oil) scenarios. All 3 species avoided medium (35, 18, 10% of trial time, respectively) and high concentrations of fresh oil (30, 20, and 15%, respectively), while time spent over contaminated sediments at low concentrations of fresh oil was higher (30, 40, and 40%, respectively). Weathered crude elicited no significant avoidance behavior, with fish occupying between 40 and 60% of the trial period over these sediments, regardless of concentration. This research highlights the heretofore unrecognized role of behavior in fish resilience, as well as the need for future studies to incorporate ecologically relevant weathering rates. Such results are critical to the successful management of motile resources, such as estuarine fishes, in response to anthropogenic disasters such as oil spills.

**KEY WORDS:** Salt marsh · Gulf of Mexico · Fish ecology · Experiment · Avoidance behavior · Oil contamination · Oil spill · *Deepwater Horizon* · Macondo

## INTRODUCTION

The April 2010 explosion and sinking of the *Deepwater Horizon* (DWH) drilling platform off the coast of Louisiana (USA) in the Mississippi Canyon Block 252 (MC252) released about 5 million barrels of 'sweet' Louisiana crude oil into surrounding waters as it flowed unabated for 87 d (Crone & Tolstoy 2010). It has been recognized as the largest marine oil spill in US history, equivalent to 20 times the areal coverage of the 'Exxon Valdez' disaster (Turner et al. 2014a). Prevailing meteorological conditions led to the subsequent oiling of many coastal wetlands rang-

ing from Louisiana to the Florida Panhandle in the northern Gulf of Mexico (GoM), with a total of 1773 km of affected shoreline (Michel et al. 2013).

The oil released during this event was a complex mixture containing tens of thousands of different chemical constituents, ranging from lightweight aromatic chemicals that volatilize and degrade quickly, to larger carbon chains that are resilient to weathering. As oil was distributed into inshore coastal areas, numerous processes (including photooxidation, evaporation, emulsification, microbial activity, dissolution, and adsorption to sediment particles) led to a much different chemical mixture than that found closer to

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the offshore source (Mendelsohn et al. 2012). In general, oil reaching inshore areas was heavily weathered, and this altered crude was less toxic with fewer water-soluble compounds (Reddy et al. 2012).

The various habitats (marshes, mangroves, sandy beaches, and seagrass beds) in the affected area provide a variety of important ecosystem services, including buffering coastlines from high-energy storm events, improving water quality and clarity, and providing food and refuge for many commercially and recreationally important organisms (Baltz et al. 1993, Peterson & Turner 1994, Rozas et al. 2013). *Spartina alterniflora* in salt marshes is the most abundant emergent plant species in coastal Louisiana, despite areal coverage decreasing from historical levels due to a variety of proposed factors, including subsidence, canalization, eutrophication, shunting of sediment supply, urbanization, and environmental toxins such as oil (Silliman et al. 2012). Salt marshes were the most frequently oiled habitat (45%) during the DWH spill, and remedial activities occurred on <9% of the affected area (Michel et al. 2013). The resilience of these habitats is critical to the persistence of resident fishes that inhabit these marshes, as well as the energy that is diverted to pelagic food webs (Peterson & Turner 1994, McCann et al. in press). The vulnerability of estuarine ecosystems and their fauna to oil released from DWH has been illustrated by numerous studies (e.g. Silliman et al. 2012, Fodrie et al. 2014, Rozas et al. 2014, Pezeshki & Delaune 2015).

While the full scope of the ecological impacts of oil in estuaries is yet to be determined, a number of studies have documented the impact to the fishes that reside in these areas. Field studies have documented the effects of DWH oil on resident fishes throughout the northern GoM (Fodrie & Heck 2011), including in Louisiana (Able et al. 2015), Mississippi (Schaefer et al. 2016), and Alabama (Moody et al. 2013). Results of these studies have overwhelmingly identified few drastic population or community changes (Fodrie et al. 2014), and even some increases in catch-per-unit-effort post spill (Fodrie & Heck 2011, Schaefer et al. 2016). Notably, very few negative impacts have been documented for resident fauna, with the exception of a short-term decline in goby biomass/density that rebounded after 1 yr in coastal Alabama (Moody et al. 2013). These results mirror those of previous oil spills in the GoM, with constant or increasing densities and no change in community structure after oiling by smaller spills in Texas (Rozas et al. 2000) and Barataria Bay, Louisiana (Roth & Baltz 2009).

Despite this consistent lack of effects in the field, a number of laboratory experiments have documented

the negative effects of oil on fishes (Fodrie et al. 2014), with deleterious impacts ranging from genomic (Garcia et al. 2012, Whitehead et al. 2012, Dubansky et al. 2013) to morphological alterations (de Soysa et al. 2012, Incardona et al. 2013). A substantial focus of previous studies has been the effects of oiling on early developmental stages of fish, as they are more sensitive than adults. The greater sensitivity of larval fishes is related to their size and lack of development (resulting in thin membranes and poorly developed systems for detoxification) and their pelagic lifestyle, which increases the risk of oil exposure. However, oil can still have considerable negative effects on adult organisms, with documented examples of reduced foraging efficiency (Gregg et al. 1997), impaired swimming behavior (Claireaux et al. 2004), and other potential indirect effects such as shifts in diet (Brzorad & Burger 1994) and delayed effects, such as the collapse of the Pacific herring population 4 yr after the Exxon Valdez oil spill (Thorne & Thomas 2008).

The dichotomy in results to date, with consistent negative effects at the individual level that fail to translate to population and community levels (Fodrie et al. 2014), could be explained by a number of potential mechanisms. For example, weathered oil reaching estuaries was often patchily distributed (Michel et al. 2013) and may have been below toxic levels. Therefore, fishes may have been able to survive and continue to reproduce despite experiencing some sublethal effects. For those organisms that did succumb to oil toxicity, many marine fishes have strong compensatory responses, especially short-lived fishes with high reproductive capabilities, such that affected areas may quickly be colonized by recruits from nearby, unaffected areas (Myers et al. 1999).

To date, one undocumented aspect that may lend resiliency to estuarine fishes is behavior and the capacity of fishes to detect and avoid oil. Here, the results of a series of experiments designed to test whether 3 common inhabitants of inshore GoM ecosystems demonstrate oil avoidance behavior are presented. Specifically, laboratory choice experiments were used to test: (1) whether species-specific patterns in behavior exist, and (2) avoidance patterns for unoiled versus a range of fresh and weathered oil concentrations.

## MATERIALS AND METHODS

### Study organisms

Gulf killifish *Fundulus grandis*, sheepshead minnow *Cyprinodon variegatus*, and sailfin molly *Poe-*



*cilia latipinna* were selected for inclusion in this study due to their abundance and widespread distribution in coastal salt marshes, seagrass beds, mangroves, and unvegetated shallow habitats throughout the GoM. Able et al. (2015) reported *F. grandis* to be the most abundant catch collected in minnow traps following the DWH oil spill, with highest abundances in marsh creeks, while *C. variegatus* and *P. latipinna* were among the 5 most abundant marsh fishes, especially in marsh ponds (>65% of the catch) and depressions (>37%). In addition, *F. grandis* is commonly used as a sentinel species and a frequent model organism in toxicology studies, along with *C. variegatus* (Able et al. 2015, Fodrie et al. 2014, Dasgupta et al. 2016, Raimondo et al. 2016). All organisms used in this study were adults collected from unoiled marshes near Louisiana Universities Marine Consortium (LUMCON) and consistent in size (total lengths: *F. grandis* 64–102 mm; *C. variegatus* 41–60 mm; *P. latipinna* 42–62 mm). All fishes were released back into the wild after the study.

### Experimental setup

Oil used in the experiments was BP Surrogate Oil, obtained from the Marlin Platform near the site of the DWH drilling platform. This oil has almost identical toxicity/chemistry as that of the crude oil released during the DWH spill (Martin et al. 2015). Prior to experiments, oil was weathered by aerating it in a volumetric flask in a fume hood to 40% by weight, a weathering representative of much of the oil that came ashore (E. Overton pers. comm.). Sediment collected from unoiled areas near Lake Pontchartrain, Louisiana, was used and, when applicable, mixed homogeneously with oil, placed on 1 randomized side of a 38 l tank containing an airstone, and filled with 10 psu water mixed using Instant Ocean™ salt and dechlorinated tap water.

Tanks were randomly assigned oil concentrations (low, medium, or high) and weathering (unweathered or 40% weathered) such that each fish species had a choice between no oil and each unique concentration and condition of oil (no oil vs. unweathered low oil, no oil vs. unweathered medium oil, no oil vs. unweathered high oil, no oil vs. weathered low oil, no oil vs. weathered medium oil, no oil vs. weathered high oil). The concentrations of oil used in the experiment were: 0, 10, 20, and 40 ml oil l<sup>-1</sup> of sediment for the no oil and the low, medium, and high oil treatments, respectively. These concentrations span the range of oiling found in field studies (Turner et al.

2014a,b) and were used in previous experimental assessments (Horel et al. 2012, Martin et al. 2015). All treatments were replicated (n = 10), and no individual was used more than once in trials. A randomized sample of sediment was taken from each unique treatment level and analyzed for alkane and aromatic oil concentrations to verify treatments following methods described by Turner et al. (2014a,b).

During trials, 1 fish was released into the tank and allowed to acclimate for a period of 5 min. Its movements between the 2 'habitats' (no oil vs. respective treatments) were then recorded using a GoPro digital camera (1040HD) for a period of 10 min. These experimental periods are similar to those used in other studies of fish behavior (Gerlach et al. 2007, Paris et al. 2013, Martin 2017). The time spent on each side of the tank was documented and compared among treatments. A series of trials with no oil on either side were also conducted and indicated no preference for a particular side of the tank; these data were not included in further analyses.

### Statistical analyses

A general linear model was created, using time spent in the side of the tank containing oil as a response variable and oil concentration (low, medium, high), weathering (unweathered, weathered), and species (*F. grandis*, *C. variegatus*, or *P. latipinna*) as factors. When significant differences were detected, a Tukey's post hoc test was performed. Comparisons of habitat preference were made by assuming a 1:1 occupancy in each habitat and testing whether the time spent on each side of the tank varied significantly using a paired *t*-test (Peterson & Renaud 1989, Martin & Valentine 2011). Prior to all analyses, normality and homogeneity of variance were tested and transformations were made, if necessary. Nonparametric alternatives were used if transformations failed to meet assumptions of the tests. Results were considered significant at  $p < 0.05$ , and highly significant at  $p < 0.01$ .

## RESULTS

Sediment alkane (Fig. A1 in the Appendix) and aromatic (Fig. A2) concentrations confirmed the presence of various oil compounds across treatments. Specifically, fresh oil contained total alkane concentrations of 25 510, 4236, 3292, and 0.856  $\mu\text{g g}^{-1}$  for high, medium, low, and no oil, respectively, while

weathered oil contained 5349, 3659, 2730, and  $0.735 \mu\text{g g}^{-1}$ , respectively. Likewise, total aromatic concentrations varied across treatments with fresh oil containing more (high: 1561, medium: 596, low: 274, no oil:  $0.014 \mu\text{g g}^{-1}$ ) than weathered oil (high: 468, medium: 238, low: 185, no oil:  $0.357 \mu\text{g g}^{-1}$ ).

Response of fishes to the presence of oil was consistent among the 3 species tested here (Fig. 1;  $F_{2,162} = 0.60$ ,  $p = 0.549$ ). However, avoidance behavior was significantly different among the different concentrations of oil ( $F_{2,162} = 3.97$ ,  $p = 0.021$ ), with medium and high concentrations usually eliciting a stronger response than low concentrations (Fig. 1). Oil weathering also had a highly significant effect ( $F_{1,162} = 46.37$ ,  $p < 0.001$ ) on avoidance, with greater response to fresh oil than weathered oil. No interactive effects were detected (species  $\times$  oil concentration  $F_{4,162} = 0.66$ ,  $p = 0.169$ ; species  $\times$  oil weathering  $F_{2,162} = 2.19$ ,  $p = 0.115$ ; oil concentration  $\times$  oil weathering  $F_{2,162} = 2.05$ ,  $p = 0.133$ ; species  $\times$  oil concentration  $\times$  oil weathering  $F_{4,162} = 0.56$ ,  $p = 0.695$ ).

Individual comparisons between time spent in oil or no oil for each choice test highlighted important differences in fish behavior. For example, weathered crude had no effect on the habitat occupancy patterns for any of the 3 fish species, with the proportion of

Table 1. Paired  $t$ -test statistics for each species and comparison. Low, medium, and high oil concentrations were 10, 20, and 40 ml oil  $\text{l}^{-1}$  of sediment, respectively

Comparison	$t$	$p$
<b><i>Fundulus grandis</i></b>		
No oil vs low oil	-2.479	0.035
No oil vs weathered low oil	0.456	0.659
No oil vs medium oil	-2.622	0.028
No oil vs weathered medium oil	-0.821	0.433
No oil vs high oil	-5.279	0.001
No oil vs weathered high oil	0.541	0.601
<b><i>Poecilia latipinna</i></b>		
No oil vs low oil	-0.904	0.390
No oil vs weathered low oil	0.0163	0.747
No oil vs medium oil	-8.152	0.001
No oil vs weathered medium oil	-0.462	0.655
No oil vs high oil	2.784	0.002
No oil vs weathered high oil	0.817	0.435
<b><i>Cyprinodon variegatus</i></b>		
No oil vs low oil	-1.836	0.100
No oil vs weathered low oil	1.259	0.240
No oil vs medium oil	2.963	0.001
No oil vs weathered medium oil	-0.768	0.462
No oil vs high oil	-8.001	0.001
No oil vs weathered high oil	0.907	0.388

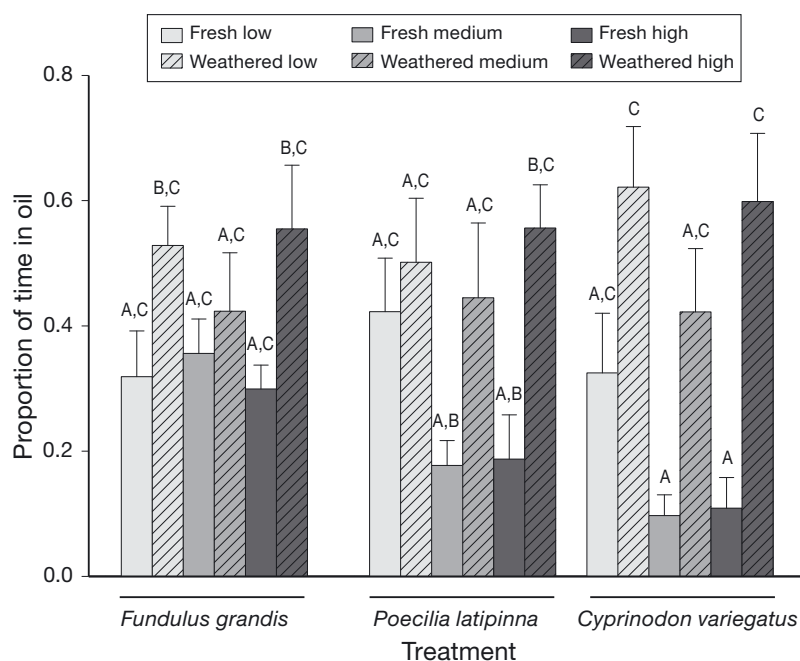


Fig. 1. Comparison of time spent in the side of the tank containing oil among fish species and oil concentrations and degree of weathering. Light grey, grey, and dark grey indicate low, medium, and high oil concentrations, respectively. Solid bars represent fresh oil; hatched bars show weathered oil. Different letters indicate statistically significant differences ( $p < 0.05$ )

time in oil ranging from 0.4 to 0.6 (Table 1, Figs. 2–4). In contrast, higher concentrations of fresh oil elicited a very strong avoidance response from all fishes tested ( $p < 0.001$ ; Table 1, Figs. 2–4). *Fundulus grandis*, *Poecilia latipinna*, and *Cyprinodon variegatus* all spent small amounts of time (about 30, 20, and 15%, respectively) over sediments with high concentrations of fresh oil. Medium concentrations of fresh oil also elicited a highly significant, very strong response ( $p < 0.001$ ) from *P. latipinna* (Table 1, Fig. 3) and *C. variegatus* (Table 1, Fig. 4) (~18 and 10% of the trial duration, respectively) and a significant response ( $p < 0.05$ ) from *F. grandis* (Table 1, Fig. 2) (~35% of the trial duration). The response of fishes to low concentrations of fresh oil were variable, however. *F. grandis* displayed significant avoidance (Table 1, Fig. 2), spending around 30% of the trial in the side of the tank containing sediment with a low concentration of fresh oil, while *C. variegatus* averaged

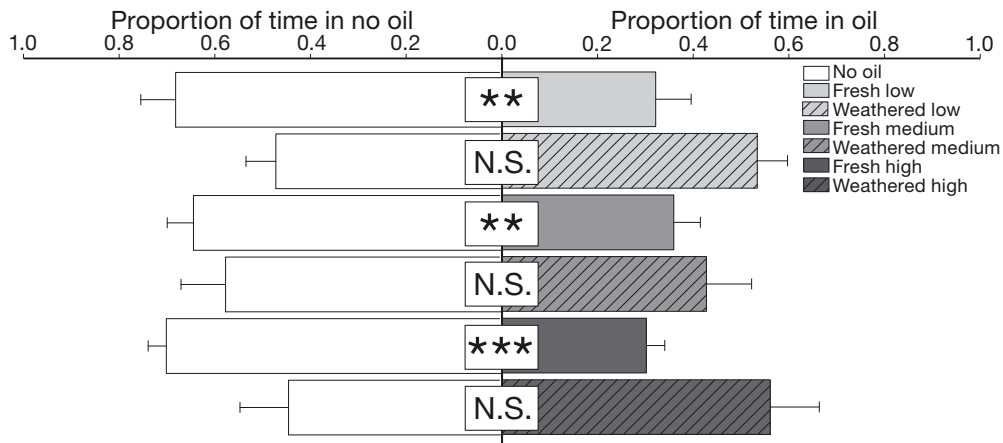


Fig. 2. Proportion of time spent in the side of the tank containing no oil (left) and oil (right) for *Fundulus grandis*. Light grey, grey, and dark grey indicate low, medium, and high oil concentrations, respectively. Solid bars represent fresh oil; hatched bars show weathered oil. NS: not significant, \* $p < 0.10$ , \*\* $p < 0.05$ , \*\*\* $p < 0.001$

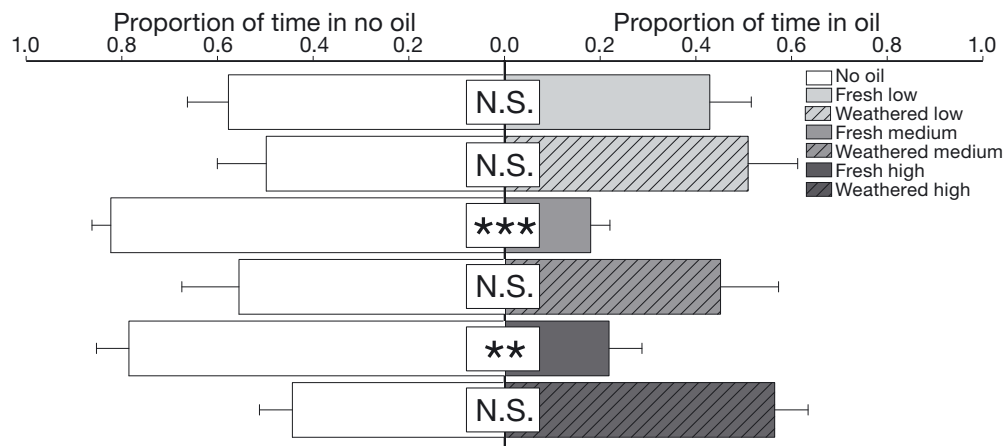


Fig. 3. As in Fig. 2, but for *Poecilia latipinna*

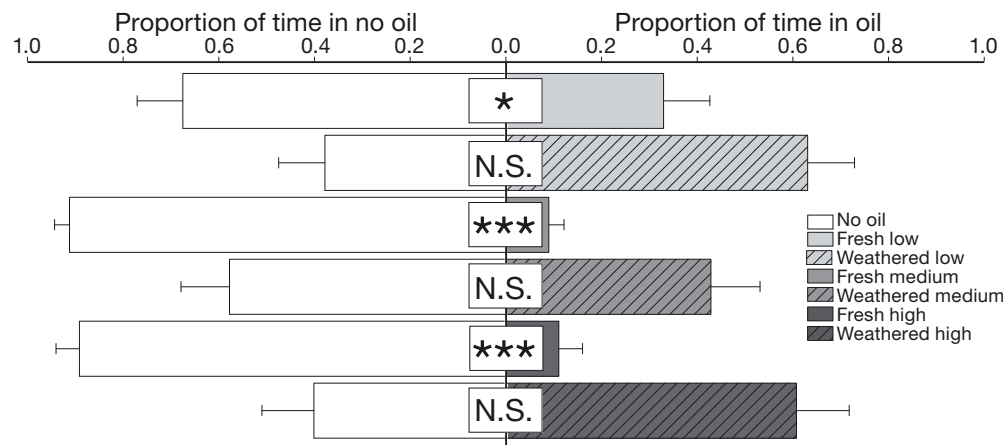


Fig. 4. As in Fig. 2, but for *Cyprinodon variegatus*



~40% of the time in oil, perhaps a biologically meaningful ( $p < 0.10$ ) avoidance (Table 1, Fig. 4). *P. latipinna* did not exhibit a response to fresh oil at low concentrations, with over 40% of the trial duration spent in the oiled portion of the tank.

## DISCUSSION

The devastating effects of the 2010 DWH disaster included loss of human and marine life, as well as negative effects on coastal ecosystems such as acceleration of marsh loss (Silliman et al. 2012). While some negative effects on fishes have been reported offshore (Murawski et al. 2014) and in laboratory studies on inshore fishes, the consequences for many coastal fishes remain uncertain. Although controlled manipulations of oil demonstrate significant negative physiological effects, surveys of communities and populations in affected areas have yet to demonstrate long-term consequences of these individual-level effects (Fodrie et al. 2014).

Results presented here demonstrate that these estuarine fishes exhibit strong aversions for fresh oil at medium and high concentrations. However, at low concentrations of fresh oil, only *Fundulus grandis* responded significantly. In contrast, fishes in choice trials with weathered oil did not display significant preference patterns at any concentration. Given the range of oil constituents found in nearshore ecosystems such as salt marshes (Turner et al. 2014a,b), these results highlight the need for a more detailed and ecologically relevant assessment of laboratory assays, as many controlled experiments to date only use fresh, unweathered oil to make predictions regarding the effects of oil in the field. In addition, laboratory experiments measuring physiological and morphological responses may overestimate field responses due to these experimental artifacts (but see Whitehead et al. 2012). As such, the discrepancy in studies to date could be due in part to organism avoidance of freshly oiled, and hence more toxic, sediment.

The origin of oil offshore in Louisiana allowed considerable time and opportunity for enhanced weathering and ultraviolet degradation of oil before it reached nearshore ecosystems. When oil finally arrived onshore, the more toxic chemicals (such as naphthalenes) had already precipitated, leaving the more stable, longer carbon chains that dissolve less readily into the water and are less toxic to nekton (Reddy et al. 2012, Turner et al. 2014a,b). As such, it is plausible that fish use these most toxic compounds

as a cue to guide avoidance behavior. Future research should focus on isolating individual constituents of oil that may trigger the responses observed in the current study. Further, it is unclear whether skin irritation from aromatic compounds, or olfactory detection of oil drives the behavioral patterns documented here, and future research should be directed at determining the relative roles of each mechanism.

These results point to the importance of conducting oil studies at relevant concentrations and degrees of weathering. In nearshore areas, crude oil is usually highly weathered and less toxic to fishes (although it is important to note that no mortality was detected in these trials, even with fresh oil) (Reddy et al. 2012). Unweathered crude, however, may be found in coastal areas in the center, protected portion of tar balls, and bound to sediments in anoxic areas where degradation is slower (Mendelssohn et al. 2012).

Contaminants, such as petroleum hydrocarbons, have long been known to alter animal behavior (Weis et al. 2001). Oil-induced avoidance behavior has been documented in a number of aquatic organisms. At very small scales, calanoid copepods consistently alter their swimming behavior to avoid patches of water-soluble fractions of diesel oil, suggesting that behavioral capacities could minimize exposure (Seuront 2010). Likewise, acoustic recordings have confirmed that sperm whales that historically used the area near the DWH spill site relocated to a site farther away from the oiled area (Ackleh et al. 2012). Other marine mammals such as dolphins are known to avoid oil mousse (Smultea & Würsig 1995), and have even been trained to detect oil via olfactory mechanisms (Geraci et al. 1983).

Previous studies have also documented the response of fishes to oil contamination. For example, Farr et al. (1995) manipulated concentrations of fluoranthene, a toxic polycyclic aromatic hydrocarbon, and found that fathead minnows *Pimephales promelas* avoided contaminated waters down to  $8.6 \mu\text{g l}^{-1}$ . Similarly, both striped bass *Morone saxatilis* and rainbow trout *Oncorhynchus mykiss* avoided effluent from a California oil refinery (Carr et al. 1990). In the marine environment, juvenile flatfishes (Moles et al. 1994) and juvenile spot *Leiostomus xanthurus* (Hinkle-Conn et al. 1998) avoided heavily oiled sediments, but not lightly oiled areas.

Given that the fishes included in this study did not avoid weathered crude or (in some cases) low concentrations of fresh oil, a rich field of future study includes the sublethal, indirect effects of oil on fauna. Some organisms, such as fiddler crabs and terrestrial

arthropods, exhibited an initially negative response to oil that came ashore (McCall & Pennings 2012). However, with less water-soluble compounds to affect organisms at higher weathering rates (Mendelssohn et al. 2012), which comprised the bulk of the oil that came ashore (Reddy et al. 2012), coupled with the lack of strong population/community responses (Fodrie et al. 2014), it is likely that fishes in affected areas were exposed to crude oil at sublethal levels.

To date, several studies have noted the sublethal consequences of oil. Field mesocosms in a range of contaminated areas found that penaeid shrimps grow more slowly in heavily oiled areas (Rozas et al. 2014). Specifically, a 60% decrease in brown shrimp *Farfantepenaeus aztecus* daily growth rate was observed after only 7 d. While varying sensitivities to contamination may influence food sources (Brzorad & Burger 1994, Thorne & Thomas 2008), oil also reduces foraging activity. Foraging rates of darter gobies *Gobionellus boleosoma* were reduced by 50 to 100% in the presence of diesel-contaminated sediments, but not at low concentrations (Gregg et al. 1997). Likewise, spot exhibited decreased feeding strikes within 30 min during feeding trials in sediments contaminated with diesel fuel (Hinkle-Conn et al. 1998). A broader understanding of these sublethal effects in salt marsh ecosystems is needed to fully assess food web alterations in the wake of the DWH spill.

The results of this study contribute significantly to our understanding of estuarine fish resilience to disasters such as oil spills. Behavior likely plays a key role in fish persistence in nearshore environments such as salt marshes. However, it is noteworthy that many fishes may not exhibit such behavior and may not be as resilient to these contaminants. Marsh fishes, such as those used here, are exposed to a wide range of environmental conditions including extreme fluctuations in temperature and dissolved oxygen, and as a result may be more tolerant of stressful conditions. The fauna and flora of Louisiana have been exposed to oil for centuries through natural seeps and human exploitation. Therefore, it is possible that exposure may have been a selecting force for fishes exhibiting such behavioral responses, but future research needs to confirm this. This study establishes important baseline information regarding the behavior of fishes exposed to these contaminants, with critical implications for the continued management of coastal ecosystems.

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Research Initiative Information Data Cooperative (GRIIDC) at <https://data.gulfresearchinitiative.org> (doi:10.7266/N7NV9G95). This manuscript was inspired and has been greatly improved through discussions with K. Able, J. Fodrie, O. Jensen, P. Lopez-Duarte, B. Roberts, E. Turner, and J. Valentine and the constructive criticisms of anonymous reviewers.

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**Appendix.** Sediment oil concentrations used in behavior experiments

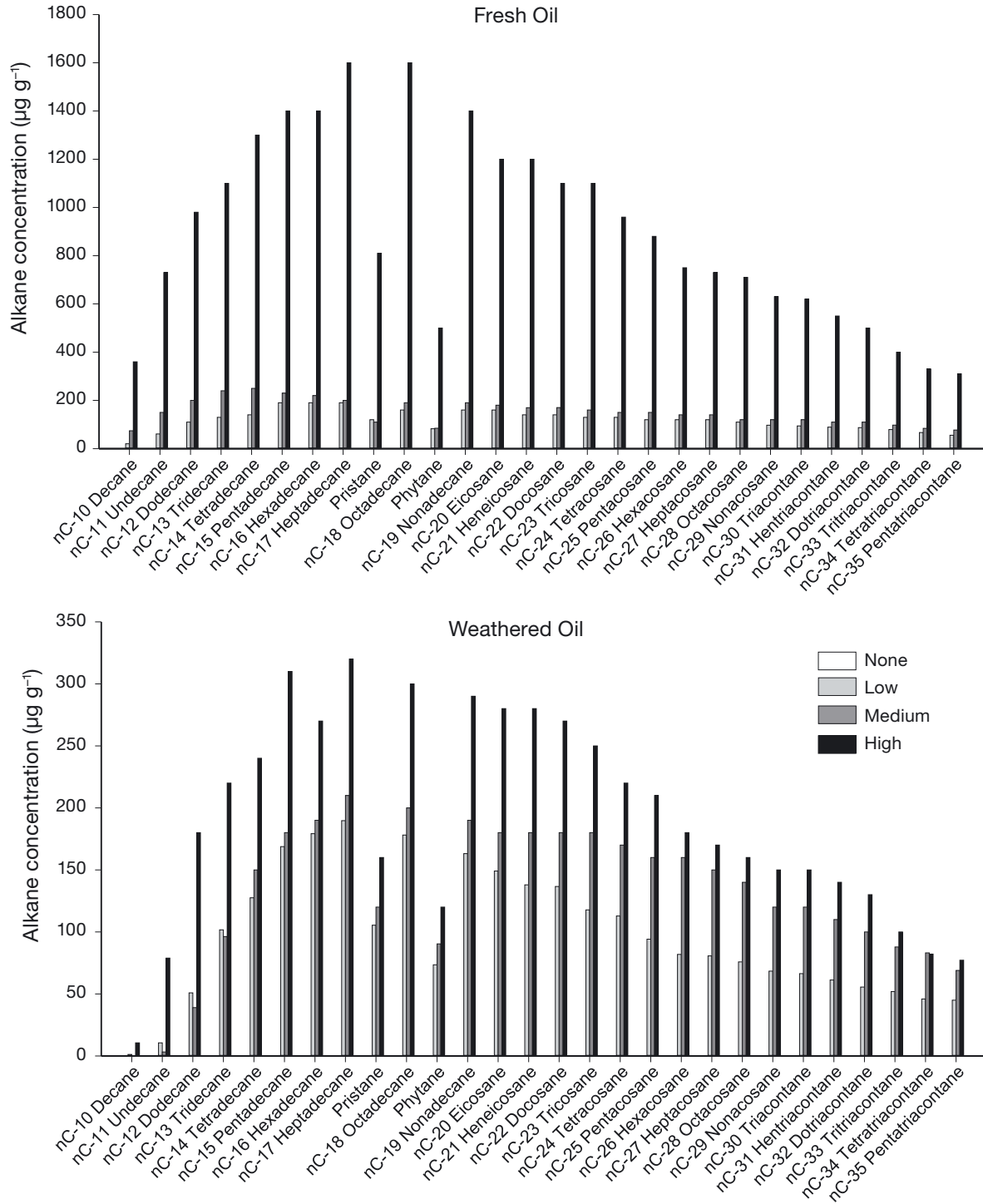


Fig. A1. Alkanes measured in sediment of each treatment for fresh and weathered oil trials (where low, medium, and high oil concentrations were 10, 20, and 40 ml oil l<sup>-1</sup> of sediment)

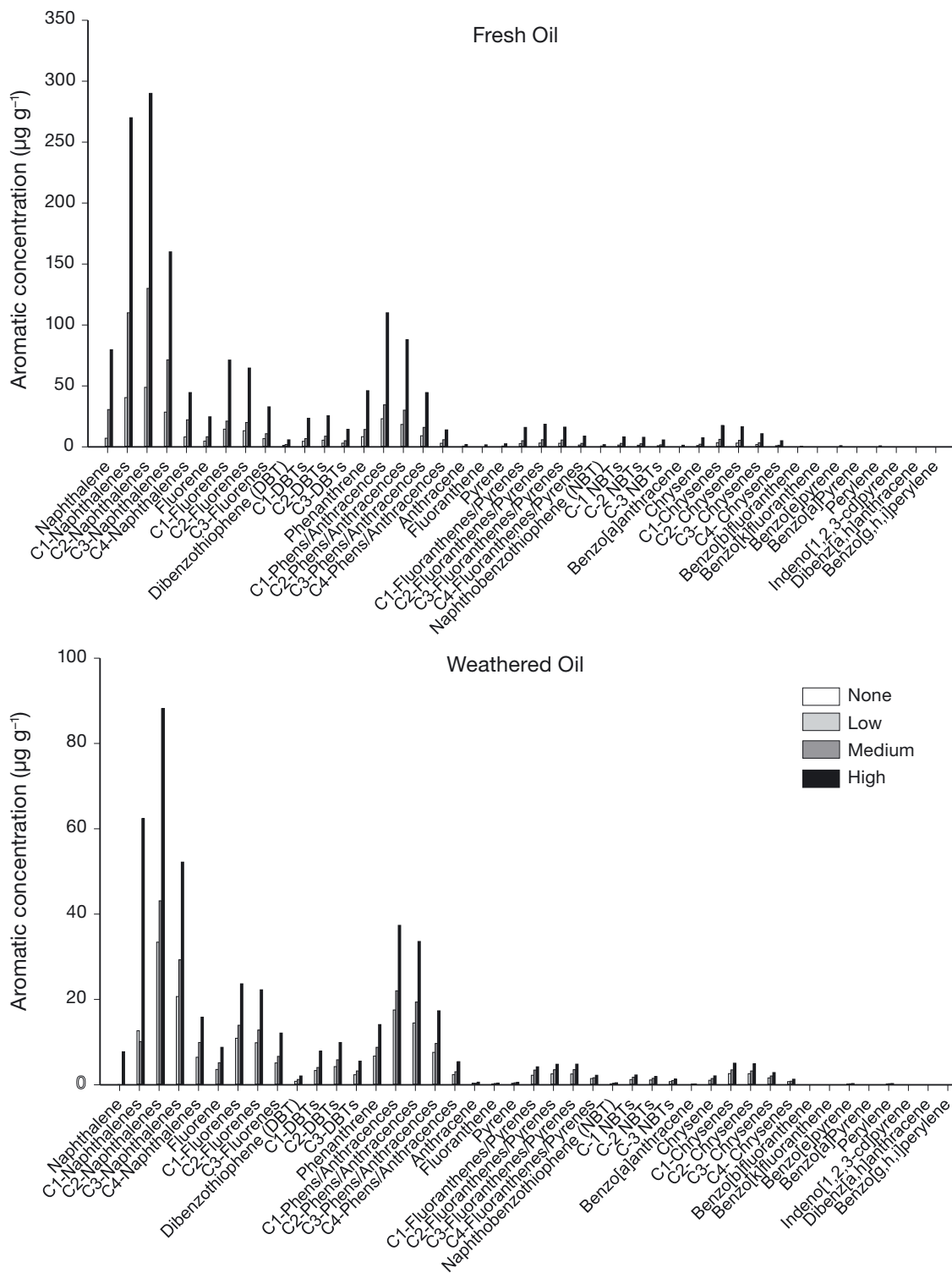


Fig. A2. Aromatics measured in sediment of each treatment for fresh and weathered oil trials (where low, medium, and high oil concentrations were 10, 20, and 40 ml oil l<sup>-1</sup> of sediment)



Contribution to the Theme Section

'Response of nearshore ecosystems to the Deepwater Horizon oil spill'



# Five years of *Deepwater Horizon* oil spill effects on marsh periwinkles *Littoraria irrorata*

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**ABSTRACT:** The *Deepwater Horizon* spill (2010) was the largest marine oil spill in US waters to date and one of the largest worldwide. To examine effects of the oil spill on an important salt marsh species over time, we conducted a meta-analysis on marsh periwinkles *Littoraria irrorata* using published and unpublished sources spanning more than 5 yr (2010–2015), including newly available Natural Resources Damage Assessment (NRDA) and Gulf of Mexico Research Initiative (GoMRI) data sets. We tested the hypotheses that the spill decreased mean periwinkle density, reduced mean snail shell length, and changed periwinkle size distribution. Averaged across multiple studies, sites, marsh zones (edge versus interior), and years, our synthesis revealed a negative effect of heavy oiling on periwinkles. Snail densities were reduced by 73% in heavily oiled sites across all study-zone-by-year combinations, including adverse effects for both the oiled marsh edge and oiled marsh interior, with impacts observed over more than 5 yr. Mean periwinkle shell length was somewhat reduced at the oiled marsh edge in a few cases; however, periwinkle size distributions displayed greater relative proportions of smaller adults and sub-adults, and fewer large adults, across all years. Given the spatial and temporal extent of data analyzed, this synthesis provides evidence that the *Deepwater Horizon* spill suppressed populations of marsh periwinkles in heavily oiled marshes for over 5 yr, and that impacts were ongoing and recovery was incomplete, likely affecting other ecosystem components, including marsh productivity, organic matter and nutrient cycling, marsh–estuarine food webs, and associated predators.

**KEY WORDS:** *Deepwater Horizon* · Oil spill · Salt marsh · Marsh periwinkle · *Littoraria irrorata* · Ecological impacts · Ecological recovery · Gulf of Mexico

## INTRODUCTION

The *Deepwater Horizon* oil spill (2010) was the largest marine oil spill in US waters to date and one of the largest worldwide: over 3 million barrels of

crude oil were released into the Gulf of Mexico (US District Court 2015) and over 2100 km of coastal shorelines were oiled (Michel et al. 2013, Nixon et al. 2016). The impacts of this oil spill on salt marsh vegetation have been well documented (Lin & Mendels-

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sohn 2012, Silliman et al. 2012, Zengel et al. 2015, 2016a, Lin et al. 2016, Hester et al. 2016). In comparison, the impacts of this spill on salt marsh macroinvertebrates have received less attention (although see Zengel et al. 2016a, a single-year Natural Resources Damage Assessment [NRDA] study on marsh periwinkles; and Zengel et al. 2016b on fiddler crabs [*Uca* spp.]). As a further step in understanding the effects of this oil spill on salt marsh macroinvertebrates, we assessed multi-year effects of the *Deepwater Horizon* oil spill on marsh periwinkles *Littoraria irrorata*, the dominant gastropod (snail) species in salt marshes in the Gulf region, and an important secondary consumer and prey species.

Marsh periwinkles are abundant salt marsh residents and have important influences on marsh vegetation, organic matter and nutrient cycling, microbial communities, other invertebrates, and ecosystem productivity (Zengel et al. 2016a and references therein). For example, periwinkle grazing plays a key role in the shredding and decomposition of senescent and standing dead *Spartina alterniflora* plant leaves, thereby influencing organic matter and nutrient cycling and marsh–estuarine food webs (Newell et al. 1989, Kemp et al. 1990, Hensel & Silliman 2013). Periwinkle grazing may also regulate plant productivity, and in some cases may contribute to marsh vegetation die-back events (Silliman & Zie-man 2001, Silliman et al. 2005). Marsh periwinkles are also important prey for many species of commercial, recreational, and conservation interest, including blue crab *Callinectes sapidus*, diamondback terrapin *Malaclemys terrapin*, clapper rail *Rallus longirostris*, and perhaps seaside sparrow *Ammodramus maritimus* (Hamilton 1976, Heard 1982, Tucker et al. 1995). Accordingly, oil spill impacts on periwinkles could affect overall marsh structure and function and a variety of other marsh and estuarine species.

A few studies have documented impacts on marsh periwinkles following prior oil spills, with effects including increased mortality, reduced density, reduced recruitment, and altered snail size distributions (Hershner & Moore 1977, Hershner & Lake 1980, Krebs & Tanner 1981, Lee et al. 1981). However, each of these spills differed from the *Deepwater Horizon* incident in several important ways: (1) all were located outside the Gulf of Mexico region; (2) all were shallow, nearshore spills or experimental oil applications that rapidly affected shorelines, whereas oil from the offshore *Deepwater Horizon* release weathered at sea for 2 wk or more before reaching shore; (3) all were spills of refined products, including No. 6 and No. 2

fuel oils, which differ from crude oils in terms of their chemistry and ecological fate and effects (Michel & Rutherford 2014); and (4) all affected relatively small areas of salt marsh compared to the widespread shoreline oiling observed during the *Deepwater Horizon* event. These differences could result in varying ecological responses to oiling.

Five published studies have reported on the effects of the *Deepwater Horizon* oil spill on marsh periwinkles (McCall & Pennings 2012, Silliman et al. 2012, Zengel et al. 2014, 2015, 2016a). However, each of these studies either primarily focused on other topics (e.g. insects, vegetation, erosion), or was limited in timing, duration, number of sites studied, or specific locations and conditions examined relative to marsh periwinkles. Zengel et al. (2016a) was a detailed treatment of periwinkle impacts spanning a large number of sites, a relatively large area of oiled shoreline, and both the marsh edge and interior. However, that study only covered a single year of sampling (2011), thereby limiting the ability to examine longer-term periwinkle impacts and recovery. Because natural populations can be quite variable in space and time, it can be difficult in many cases for individual studies to clearly identify population and community changes even after large oil spills, hence the need for integration of data across multiple studies (Fodrie et al. 2014). Here, we synthesize results of these 5 studies and other ongoing work in a meta-analysis, using published and unpublished sources spanning more than 5 yr, including newly available Natural Resources Damage Assessment (NRDA) and Gulf of Mexico Research Initiative (GoMRI) (Deis et al. 2015) data. We tested the hypotheses that the oil spill reduced periwinkle density (snail abundance), reduced mean periwinkle shell length (snail size), and altered periwinkle size distributions in salt marshes that were heavily oiled. Using these results, we also examined prior projections of periwinkle recovery time frames based on studies published to date (see Zengel et al. 2014, 2015, 2016a).

## METHODS

### Studies

We synthesized all published periwinkle data comparing heavily oiled and reference sites collected after the spill (April 2010) and additional unpublished data, including large NRDA and GoMRI data sets (Table 1). Detailed methods are included in each of the published studies and are available for the



Table 1. Summary of marsh periwinkle data sources used in the meta-analysis. Zones 1, 2, and 3 refer to the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of heavy oiling, respectively. NRDA: Natural Resources Damage Assessment

Source	Years	Zones	Density	Shell length	References/Notes
Silliman	2010–2013	1–2	X	–	Silliman et al. 2012, including unpubl. data
Pennings	2010–2011	3	X	–	McCall & Pennings 2012
NRDA	2011	1–3	X	X	Zengel et al. 2016a
Zengel	2011–2015	1	X	X <sup>a</sup>	Zengel et al. 2014, 2015, including unpubl. data
Deis	2012–2015	1	X	X	Deis et al. 2015, including unpubl. data

<sup>a</sup>2012–2015 only

GoMRI data (Deis et al. 2015). Study sites were widely distributed throughout the northern Gulf of Mexico (Louisiana, Mississippi, Alabama), with numerous sites concentrated in southeastern Louisiana (Barataria and Terrebonne Bays), where salt marsh oiling was most widespread and severe (Fig. 1). No known studies were excluded from our analyses.

Sites were categorized as oiled or reference by the primary investigators using somewhat different criteria, but all the studies described visibly oiled sites with heavy oiling. The vast majority of the oiled sites corresponded to the NRDA ‘heavier persistent oiling’ category, defined as marsh shorelines with heavy to moderate visible oiling that persisted on the shoreline for 3 mo or longer (Nixon et al. 2016). All studies reported that their reference sites had no visible oiling or impacted vegetation at the time data were collected.

Some of the oiled sites had active, intensive shoreline cleanup treatments applied as part of the *Deep-water Horizon* emergency response, involving man-

ual and mechanical removal of oiled wrack, raking and cutting of oiled marsh vegetation, and raking and scraping of oil deposits from the marsh substrate (Zengel et al. 2015, 2016a)—note that all or nearly all oiled sites likely had passive treatment involving sorbent boom deployment just seaward of the marsh edge, often followed by boom stranding in the marsh and subsequent retrieval operations; both oiled and reference sites may have had protective booming, including both hard and sorbent boom deployment and stranding. Because we were interested in examin-

ing the overall impacts of the oil spill, including effects of oiling and any associated shoreline treatment, and because some sources did not describe whether sites were actively treated, we pooled all oiled sites into a single category regardless of whether they were actively treated (averaged across sampling years, ~27% of oiled sites were known to have had active shoreline cleanup treatments).

Nearly all sites, reference and oiled, were located in mainland herbaceous salt marsh with muddy organic soils. A few sites were located in back barrier island salt marsh and may have had somewhat sandier soils. The salt marsh vegetation at all sites was naturally dominated by *Spartina alterniflora* and in some cases *Juncus roemerianus*, the typical salt marsh species in the region. Reference versus oiled sites within studies were compared by their respective investigators across metrics such as soils, salinity, vegetation types etc., and were found to be generally similar in terms of habitat for marsh periwinkles, other than oiling conditions and subsequent impacts on the marsh habitat (we consider 1 possible exception in the ‘Discussion’).

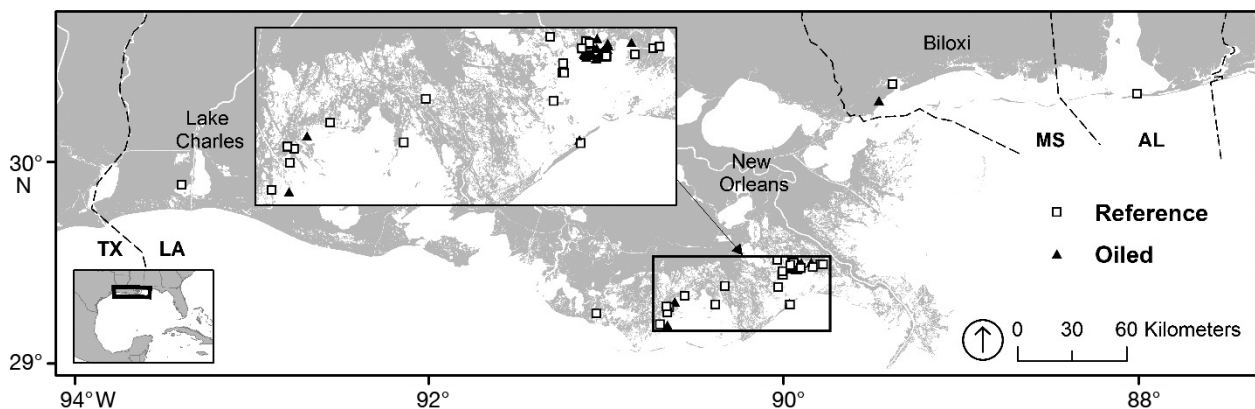


Fig. 1. Study area in the Gulf of Mexico showing locations of reference and oiled sampling sites

### Marsh zones

Oil was deposited most heavily along the seaward edge of marshes, with gross visible oiling typically limited to ~10–20 m from the shoreline, though oiling extended further into the marsh in some areas (Lin & Mendelssohn 2012, Silliman et al. 2012, Kokaly et al. 2013, Michel et al. 2013, Zengel et al. 2015, 2016a). Marsh periwinkles also naturally vary in distribution and abundance between the marsh edge and interior (Zengel et al. 2016a). We therefore divided the data into 3 'marsh zones'. Zone 1 was defined as the seaward marsh edge, where oiling was typically heaviest (~0–6 m from the shoreline, depending on oiling width across shore). Zone 2 was defined as the marsh interior within the main oiling band (~6–15 m from the shoreline, depending on oiling width). Zone 3 was defined as the marsh interior a few meters landward (inland) of the main oiling band, with light to no visible oiling. Designations for marsh edge versus marsh interior were similar to those in Peterson & Turner (1994). Due to shoreline erosion (~1–3 m yr<sup>-1</sup>), sampling position relative to distance from the shoreline was not static over time; however, even in later sampling years, Zone 1 sampling sites were still located within the shoreline widths most affected by oiling and most similar to earlier Zone 1 locations (and these sites had not yet eroded beyond the areas with heaviest oiling). In some cases, Zone 1 sites in later years may have been similar to locations that would have originally been considered Zone 2. In all cases, zones at the reference sites were located at distances similar to those of the oiled sites.

### Periwinkle density

All studies estimated periwinkle abundance based on density counts of snails on the marsh vegetation and substrate using 0.25–1 m<sup>2</sup> quadrats (reported as no. periwinkles m<sup>-2</sup>). Most, but not all, sampling was conducted in summer and early fall. Preliminary analysis showed no major indications of seasonality in the density data, so we did not exclude any sampling dates. Where one study sampled 2 or more times per year (Deis et al. 2015), we used an annual average for each site. We were able to compile density data for 22 study-zone-by-year combinations. We analyzed these data in 2 ways. First, we compared periwinkle mean density between oiled and reference sites using a *t*-test for each study-zone-by-year combination. Data were  $\log(x + 0.1)$  transformed prior to analysis to improve normality, and Welch's

statistic was used where variances were unequal. We also analyzed the untransformed data using a non-parametric Mann-Whitney *U*-test (MWU test). Second, we estimated the overall effect of the oil spill using natural log (ln; hereafter, simply 'log') response ratios, using the natural log of the ratio of mean periwinkle density at oiled sites to mean periwinkle density at reference sites for each study-zone-by-year combination ( $\ln[\text{mean density oiled}/\text{mean density reference}]$ ) (after Hedges et al. 1999, Zengel et al. 2016b). The log response ratio is zero if oiled and reference sites are identical, and negative if periwinkle densities are lower at oiled sites. We compared the log response ratio to zero across all study-zone-by-year combinations using a 1-sample *t*-test and a non-parametric Wilcoxon signed rank test. Back-calculating from the log response ratio, we determined the mean response ratio and asymmetric 95% confidence intervals calculated from the *t*-test and converted these to an estimate of mean proportional reduction in periwinkle density with oiling (with lower 95% confidence boundary). For all analyses, we defined statistical significance as  $p \leq 0.10$  based on guidance from Mapstone (1995) for balancing Type I and Type II errors during environmental impact studies. All statistical tests across the study were 1-tailed.

### Shell length

A subset of studies examined snail size based on total shell length (mm) (Table 1). We were able to compile shell length data for 11 study-zone-by-year combinations. Due to seasonal effects of snail recruitment and growth, we limited our analysis to data collected in summer and early fall. We also size-censored the data, excluding juvenile snails (<6 mm shell length) from the analysis, for 2 reasons. First, small cryptic juvenile snails hidden in leaf sheaths and furled leaves were carefully sampled in some studies but not in others. Second, episodic peaks of recruitment (many very small snails) could skew or mask post-spill comparisons of mean shell length. As we did with periwinkle density, we first compared mean shell length between oiled and reference sites using a *t*-test and an MWU test for each study-zone-by-year combination, and then estimated the effect of oiling on mean shell length across all study-zone-by-year combinations by comparing the log response ratios of shell length to zero using a 1-sample *t*-test and a Wilcoxon signed rank test.

## Size distribution

Size-frequency histograms were generated to examine size distribution at the marsh edge, based on shell length data from 9 study-zone-by-year combinations (Table 1, Zone 1 only). Data were pooled across studies by reference and oiling category in each year. Data were limited to summer and early fall sampling, but the data were not size-censored (the smallest sizes were retained in order to reveal recruitment patterns, if any). 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, sub-adults, and adults, respectively (after Bingham 1972, Hamilton 1978, Stagg & Mendelssohn 2012). We used Kolmogorov-Smirnov tests to determine whether differences in size-frequency distributions were statistically significant between oiled and reference sites.

## RESULTS

### Periwinkle density

Periwinkle densities tended to be lower at oiled sites than at reference sites for nearly all study-zone-by-year combinations (Table 2, Fig. 2). Multiple study-zone-by-year combinations had statistically significant lower periwinkle densities for the oiled versus reference sites (Table 2). No corrections of p-values were applied to address multiple tests; however, only 2 statistically significant results would have been expected by chance, but 9–11 significant results were found (regardless of which test was used, i.e. *t*-test and/or MWU test). Also, although the Zone 1 difference in 2010 reported for the Silliman data (57.6 and 0.5 snails m<sup>-2</sup> for reference versus oiled, a 99% reduction in periwinkle density for the oiled sites, *t*-test *p* = 0.13; Table 2) was not statistically significant in our analysis, likely due to small

Table 2. Marsh periwinkle *Littoraria irrorata* density. Data include response ratios (RR) (periwinkle densities for oiled sites/periwinkle densities for reference sites), proportional reduction with oiling (reduction), and significance tests for each study-zone-by-year combination. Number of observations refers to the number of reference and oiled sites within the designated year, study, and zone. Values for the number of snails m<sup>-2</sup> are means ± SE. Log (ln) response ratios (RR) less than zero indicate lower densities for oiled versus reference sites. Raw density data were log(*x* + 0.1) transformed for *t*-tests; non-parametric Mann-Whitney *U*-tests (MWU) are also included. Rows in **bold** indicate statistically significant differences at *p* ≤ 0.10 for one or both tests. Zones 1, 2, and 3 refer to the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of heavy oiling, respectively

Year	Data source		No. observations		No. snails m <sup>-2</sup>		Oiled:Reference RR			p-value	
	Study	Zone	Ref.	Oiled	Ref.	Oiled	RR	lnRR	Reduction	<i>t</i> -test	MWU test
2010	Silliman	1	3	3	57.6 ± 37.5	0.5 ± 0.5	0.01	-4.68	0.99	0.13	0.18
2011	Silliman	1	3	3	28.0 ± 16.2	8.7 ± 7.7	0.31	-1.17	0.69	0.13	0.20
<b>2011</b>	<b>NRDA</b>	<b>1</b>	<b>9</b>	<b>23</b>	<b>33.8 ± 13.9</b>	<b>4.6 ± 1.2</b>	<b>0.14</b>	<b>-1.99</b>	<b>0.86</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>
<b>2011</b>	<b>Zengel</b>	<b>1</b>	<b>5</b>	<b>19</b>	<b>37.0 ± 11.5</b>	<b>0.1 ± 0.1</b>	<b>0.00</b>	<b>-5.86</b>	<b>1.00</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>
2012	Silliman	1	3	3	48.0 ± 32.6	23.1 ± 18.0	0.48	-0.73	0.52	0.22	0.35
<b>2012</b>	<b>Zengel</b>	<b>1</b>	<b>5</b>	<b>19</b>	<b>183.2 ± 40.6</b>	<b>3.5 ± 1.0</b>	<b>0.02</b>	<b>-3.95</b>	<b>0.98</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>
2012	Deis	1	7	7	32.6 ± 13.0	27.4 ± 10.8	0.84	-0.17	0.16	0.32	0.47
2013	Silliman	1	3	3	8.9 ± 8.9	8.9 ± 1.8	1.00	0.00	0.00	0.85	0.82
<b>2013</b>	<b>Zengel</b>	<b>1</b>	<b>5</b>	<b>10</b>	<b>130.0 ± 27.4</b>	<b>4.1 ± 1.6</b>	<b>0.03</b>	<b>-3.46</b>	<b>0.97</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>
<b>2013</b>	<b>Deis</b>	<b>1</b>	<b>7</b>	<b>7</b>	<b>35.1 ± 5.8</b>	<b>29.0 ± 11.5</b>	<b>0.83</b>	<b>-0.19</b>	<b>0.17</b>	<b>0.01</b>	<b>0.02</b>
<b>2014</b>	<b>Zengel</b>	<b>1</b>	<b>5</b>	<b>10</b>	<b>97.6 ± 38.4</b>	<b>8.7 ± 2.8</b>	<b>0.09</b>	<b>-2.42</b>	<b>0.91</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>
<b>2014</b>	<b>Deis</b>	<b>1</b>	<b>7</b>	<b>7</b>	<b>62.3 ± 18.6</b>	<b>54.0 ± 16.7</b>	<b>0.87</b>	<b>-0.14</b>	<b>0.13</b>	<b>0.08</b>	<b>0.12</b>
<b>2015</b>	<b>Zengel</b>	<b>1</b>	<b>5</b>	<b>10</b>	<b>181.8 ± 72.8</b>	<b>12.2 ± 5.6</b>	<b>0.07</b>	<b>-2.70</b>	<b>0.93</b>	<b>&lt;0.01</b>	<b>0.01</b>
2015	Deis	1	7	7	97.1 ± 23.7	112.3 ± 19.5	1.16	0.14	-0.16	0.48	0.75
<b>2010</b>	<b>Silliman</b>	<b>2</b>	<b>3</b>	<b>3</b>	<b>104.5 ± 23.1</b>	<b>48.4 ± 29.7</b>	<b>0.46</b>	<b>-0.77</b>	<b>0.54</b>	<b>0.18</b>	<b>0.10</b>
<b>2011</b>	<b>Silliman</b>	<b>2</b>	<b>3</b>	<b>3</b>	<b>42.7 ± 15.0</b>	<b>18.0 ± 8.0</b>	<b>0.42</b>	<b>-0.86</b>	<b>0.58</b>	<b>0.11</b>	<b>0.10</b>
<b>2011</b>	<b>NRDA</b>	<b>2</b>	<b>11</b>	<b>24</b>	<b>95.3 ± 28.0</b>	<b>46.0 ± 10.3</b>	<b>0.48</b>	<b>-0.73</b>	<b>0.52</b>	<b>0.04</b>	<b>0.05</b>
2012	Silliman	2	3	3	10.2 ± 8.9	22.5 ± 13.8	2.20	0.79	-1.20	0.62	0.75
2013	Silliman	2	3	3	28.7 ± 8.7	40.7 ± 9.8	1.42	0.35	-0.42	0.80	0.90
2010	Pennings	3	6	5	50.8 ± 23.8	64.1 ± 18.8	1.26	0.23	-0.26	0.87	0.88
2011	Pennings	3	6	6	68.5 ± 30.8	45.5 ± 11.7	0.66	-0.41	0.34	0.52	0.37
2011	NRDA	3	11	24	81.3 ± 19.7	110.8 ± 16.5	1.36	0.31	-0.36	0.68	0.84

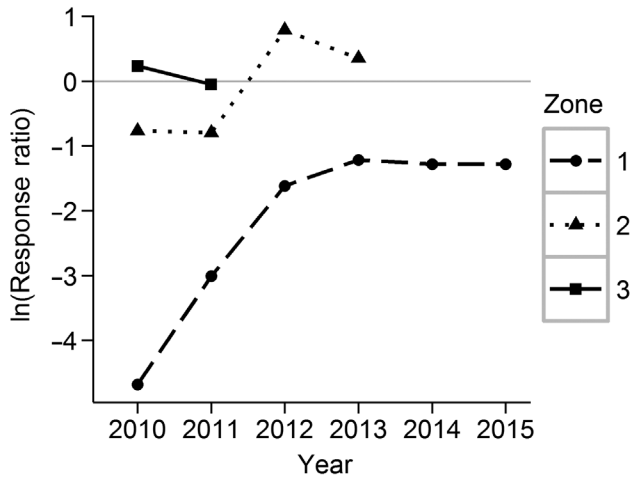


Fig. 2. Log (ln) response ratios (oiled:reference) for marsh periwinkle *Littoraria irrorata* density by zone and year. Where more than one study contributed data for any zone-by-year combination, average values were plotted. Values less than zero indicate lower periwinkle densities for oiled versus reference sites. Log response ratios were significantly lower than zero for the combined analysis across all study-zone-by-year observations (*t*-test,  $p < 0.01$ ; Wilcoxon signed ranked test,  $p < 0.01$ ). Zones 1, 2, and 3 refer to the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of heavy oiling, respectively

sample size, this difference was statistically significant in the more complex analysis conducted by Silliman et al. (2012) ( $p = 0.01$ , rounded).

As a whole, log response ratios for periwinkle density were significantly different from zero, indica-

ting that the oil spill reduced densities in the oiled sites relative to reference conditions (Fig. 2). Examining the data by zone and over time, Zone 1 differences were statistically significant across multiple studies and years, and recovery of oiled sites to reference levels was not exhibited overall (Fig. 2). Some recovery over time was observed in Zone 1; however, this leveled off and stayed below reference values during 2013–2015 (Fig. 2). Zone 2 differences were significantly different over 2010–2011 across multiple studies, with overall recovery observed by 2012 (Table 2, Fig. 2). Periwinkle densities were not affected by oiling in Zone 3 (Fig. 2). On average, periwinkle densities across all study-zone-by-year combinations were reduced by 73% in the oiled sites (95% lower confidence bound = 47% reduction).

### Shell length

Mean periwinkle shell lengths tended to be somewhat lower at oiled sites than at reference sites for more than half of the study-zone-by-year combinations, but these trends were only statistically significant for 2 comparisons (one each in Zone 1 in 2011 and 2015; Table 3, Fig. 3). For these two, mean shell lengths were 14% smaller at oiled versus reference sites (Table 3). In this case, 1 significant difference out of 11 would have been expected by chance. As a group, log response ratios for shell length were not statistically different from zero, but results did

Table 3. Marsh periwinkle *Littoraria irrorata* shell length. Data include response ratios (RR) (shell length for oiled sites/shell length for reference sites), proportional reduction with oiling (reduction), and significance tests for each study-zone-by-year combination. Number of observations refers to the number of reference and oiled sites within the designated year, study, and zone; values for shell length are means  $\pm$  SE. Log (ln) response ratios (RR) less than zero indicate smaller shell lengths for oiled versus reference sites. Raw shell length data were  $\log(x + 0.1)$  transformed for *t*-tests; non-parametric Mann-Whitney *U*-tests (MWU) are also included. Rows in **bold** indicate statistically significant differences at  $p \leq 0.10$  for one or both tests. Zones 1, 2, and 3 refer to the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of heavy oiling, respectively

Year	Data source Study	Zone	No. observations		Shell length (mm)		Oiled:Reference RR			p-value	
			Ref.	Oiled	Ref.	Oiled	RR	lnRR	Reduction	<i>t</i> -test	MWU test
<b>2011</b>	<b>NRDA</b>	<b>1</b>	<b>9</b>	<b>12</b>	<b>17.9 <math>\pm</math> 1.3</b>	<b>15.4 <math>\pm</math> 1.5</b>	<b>0.86</b>	<b>-0.15</b>	<b>0.14</b>	<b>0.09</b>	<b>0.12</b>
2012	Zengel	1	5	10	17.3 $\pm$ 0.6	16.3 $\pm$ 1.2	0.95	-0.06	0.05	0.19	0.40
2012	Deis	1	7	6	14.9 $\pm$ 1.7	16.9 $\pm$ 1.3	1.13	0.12	-0.13	0.83	0.82
2013	Zengel	1	5	8	14.2 $\pm$ 1.6	15.2 $\pm$ 2.1	1.07	0.06	-0.07	0.53	0.69
2013	Deis	1	7	7	15.9 $\pm$ 1.8	14.5 $\pm$ 1.3	0.91	-0.09	0.09	0.30	0.27
2014	Zengel	1	5	8	18.5 $\pm$ 1.1	17.9 $\pm$ 0.7	0.96	-0.04	0.04	0.34	0.18
2014	Deis	1	7	7	17.7 $\pm$ 0.7	16.7 $\pm$ 0.8	0.94	-0.06	0.06	0.17	0.19
2015	Zengel	1	5	6	19.6 $\pm$ 0.4	19.3 $\pm$ 0.8	0.99	-0.01	0.01	0.36	0.53
<b>2015</b>	<b>Deis</b>	<b>1</b>	<b>7</b>	<b>6</b>	<b>18.0 <math>\pm</math> 0.9</b>	<b>15.5 <math>\pm</math> 1.4</b>	<b>0.86</b>	<b>-0.15</b>	<b>0.14</b>	<b>0.09</b>	<b>0.09</b>
2011	NRDA	2	11	20	19.5 $\pm$ 1.1	19.8 $\pm$ 0.4	1.01	0.01	-0.01	0.64	0.34
2011	NRDA	3	11	23	20.6 $\pm$ 0.6	20.8 $\pm$ 0.2	1.01	0.01	-0.01	0.66	0.75

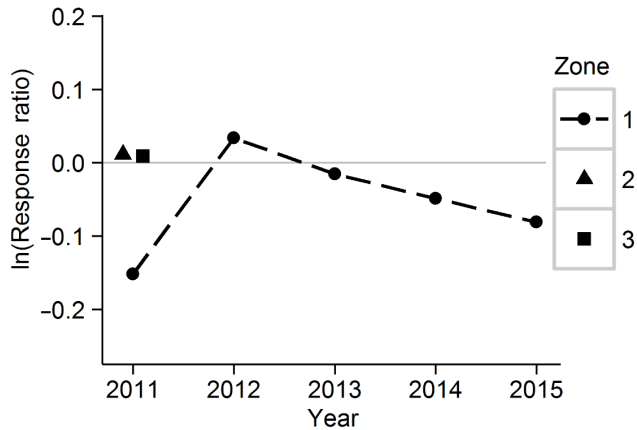


Fig. 3. Log (ln) response ratios (oiled:reference) for marsh periwinkle *Littoraria irrorata* shell length by zone and year. Values less than zero indicate smaller shell lengths for oiled versus reference sites. Log response ratios were not significantly lower than zero for the combined analysis across all study-zone-by-year observations ( $t$ -test,  $p = 0.12$ ; Wilcoxon signed ranked test,  $p = 0.12$ ). Zones 1, 2, and 3 refer to the oiled marsh edge, the oiled marsh interior, and the marsh interior inland of heavy oiling, respectively

appear to vary with time. Examining the data by zone and over time, shell length was reduced in Zone 1 in 2011, but appeared recovered by 2012 (Fig. 3). After 2012, there was a mild declining trend in shell length in Zone 1 for oiled sites compared with reference sites, with 1 record being statistically significant in 2015 (Fig. 3, Table 3). Shell size was not affected by oiling in Zones 2 or 3 (though the number of observations was limited).

### Size distribution

Periwinkle size-frequency histograms differed between the oiled and reference sites across all years and all comparisons were statistically significant (Fig. 4). Periwinkle populations in the oiled sites had greater relative proportions of smaller adults and sub-adults, and fewer larger adults, compared with reference sites (Fig. 4). This was observed across all years continuing into 2015. In addition, in several years (2012–2014) the oiled sites also had lower relative proportions of juveniles compared with reference sites, indicating low recruitment or poor survival of early recruits at the oiled sites (Fig. 4). This was most evident in 2012, when a large peak in juveniles was observed at the reference sites, but not at the oiled sites. In 2015, more juveniles were observed at oiled sites compared with reference sites; however, this may have been due to higher water levels at the time reference sites were sampled in 1 study, interfering with sampling of the smallest snails hidden in the lower leaf sheaths.

### DISCUSSION

Our analysis indicated that the *Deepwater Horizon* oil spill negatively affected marsh periwinkle populations, with ongoing effects and incomplete recovery observed more than 5 yr post-spill, especially at the oiled marsh edge. Periwinkles at oiled sites were less abundant than at reference sites. In some cases, snails

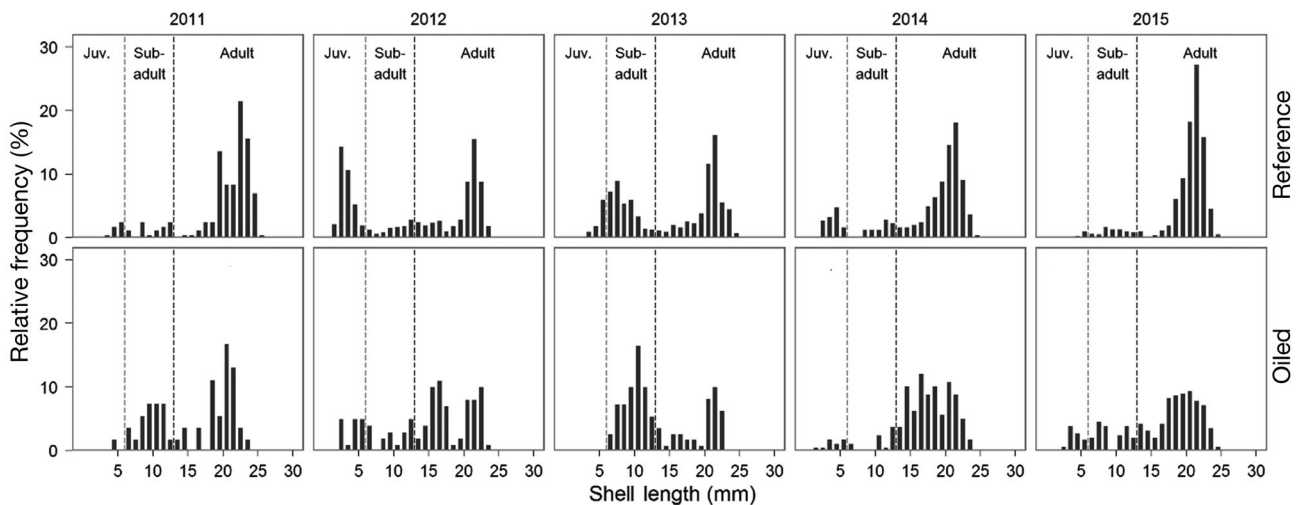


Fig. 4. Marsh periwinkle *Littoraria irrorata* size-frequency histograms for reference and oiled sites by year. Periwinkle life stage classes are defined as juveniles (<6 mm), sub-adults (6–13 mm), and adults (>13 mm). Size distributions differed between oiled and reference sites in all years (Kolmogorov-Smirnov tests,  $p < 0.01$  for each year)



were smaller on average at the oiled sites, and more clearly, overall size distribution was altered at the oiled sites across all years, with fewer large adults and lower recruitment or poor survival of new recruits in most years, compared with reference sites.

We found clear evidence that the oil spill reduced periwinkle density at the oiled marsh edge and in the oiled marsh interior across multiple years. By 2015, more than 5 yr after initial oiling, overall periwinkle density (integrated across studies) had not recovered to reference levels at the oiled marsh edge. In contrast, densities in the oiled marsh interior appeared to have recovered by 2012. Despite initial trends toward recovery, overall periwinkle density at the oiled marsh edge leveled off below reference values during 2013–2015 (short of recovery). This may indicate that periwinkle density at the marsh edge may not fully recover or that recovery could be prolonged in some instances.

Looking at individual studies over time, densities at the Silliman et al. (2012) oiled marsh edge sites appeared 'recovered' by 2013 (reference and oiled densities were similar), though their sample sizes were small and both their reference and oiled densities in 2013 were far below typical densities in the region (see Zengel et al. 2016a). Because of this, we consider recovery at the Silliman et al. (2012) marsh edge sites to be inconclusive. Density differences between oiled and reference sites in the Deis et al. (2015) data (2012–2015) were not as large as observed elsewhere, although Deis et al. (2015) indicated that a confounding influence of greater *Juncus roemerianus* plant dominance (and less *Spartina alterniflora*) may have resulted in lower periwinkle densities at their reference sites, affecting their comparisons. Even so, densities at their oiled sites appeared recovered by 2015 and were similar to mid-range to higher values typical for the region (see Zengel et al. 2016a). However, even though densities appeared recovered in the Deis et al. (2015) data, shifts in size distributions to smaller adults and sub-adults, and fewer large adults, were evident at their oiled sites across all years, similar to those observed in our combined analysis. This is likely due to initial losses of snails across all size classes, followed by subsequent recruitment or immigration of smaller (younger) snails that had not yet grown into the larger size classes. In addition, mean shell lengths in the Deis et al. (2015) data were also significantly smaller for their oiled versus reference sites in 2015. Therefore, even though periwinkle densities may have recovered in their study, population recovery in terms of size structure had not occurred in the Deis et al. (2015) sites as of 2015.

The Zengel et al. (2014, 2015) data showed the most prominent influence (largest effect sizes) on the lack of density recovery in later years, perhaps due to heavier or more persistent oiling in their study sites compared with others (see Zengel et al. 2015, Fleeger et al. 2015 for oiling conditions and comparison with the Deis et al. 2015 sites). The Zengel et al. (2014, 2015) oiled sites also displayed the same proportional shift towards smaller adult and sub-adult snails, and fewer large adults, observed elsewhere and in our overall analysis. In addition, the Zengel et al. (2014, 2015) data were the main source indicating low juvenile periwinkle recruitment or survival at the oiled sites compared to the reference sites. However, very few juvenile snails were recorded by Deis et al. (2015) at either their reference or oiled sites; this finding may have been influenced by confounding vegetation differences, overriding any possible effects of oiling on juvenile recruitment or survival (see previous paragraph). Beyond the differences and nuances of individual studies, we re-emphasize that our findings (integrated across all studies) indicate both an overall lack of periwinkle density recovery and the alteration of periwinkle size distribution through 2015 at the oiled marsh edge.

In the Zengel et al. (2016a) NRDA study, a definition of full recovery was proposed based on attaining both periwinkle density and size distributions similar to reference conditions. Zengel et al. (2016a) projected that full periwinkle recovery would take at least 3 to 5 yr once oiling and habitat conditions were suitable to support normal periwinkle life-history functions (e.g. recruitment, survival, growth). Considering our overall analyses in the present paper, and individual datasets extending into 2014–2015, we find that full recovery had yet to occur by 2015. In the case of the Deis et al. (2015) data, where *Spartina alterniflora* stem density and aboveground biomass (primary habitat constituents for marsh periwinkles; see Kiehn & Morris 2009, Stagg & Mendelssohn 2012, McFarlin et al. 2015) were reported to have recovered by 2012–2013 in their heavily oiled sites (Fleeger et al. 2015, Lin et al. 2016; both pertaining to the Deis et al. 2015 study), periwinkle size structure had still not recovered 2 to 3 yr later. This conforms to prior recovery projections, as well as observations that periwinkle recovery may depend on but lag vegetation recovery (Zengel et al. 2014, 2015, 2016a).

There are at least 3 reasons why recovery of marsh periwinkle populations might take several years. First, because residual oil remains on and in the marsh soils (Lin et al. 2016), remaining oil might continue to affect periwinkles at heavily oiled sites.

Second, because the snails depend on the marsh vegetation as their principal habitat (Kiehn & Morris 2009, Stagg & Mendelssohn 2012, McFarlin et al. 2015), snail recovery is unlikely until plant recovery is complete. Third, recruitment and immigration of new snails into the population and their subsequent growth to larger adults may be slow enough that it takes a number of years for the population to rebuild. Our findings that snail populations had not fully recovered as of 2015 is consistent with these hypotheses, and emphasizes the need for continued and long-term monitoring of populations affected by this and other environmental impacts.

Marsh periwinkles play important ecological roles in salt marshes (see Zengel et al. 2016a and references therein). They feed on and shred decomposing plant material, thereby stimulating the food web and accelerating nutrient cycling (Newell et al. 1989, Kemp et al. 1990, Hensel & Silliman 2013). When they are abundant, their feeding activity can regulate the growth of salt marsh plants (Silliman & Zieman 2001). They are important prey items for a variety of larger consumers, many of which are of commercial, recreational, and conservation importance (Hamilton 1976, Heard 1982, Tucker et al. 1995). Consequently, the long-lasting effects of the oil spill on marsh snails are likely to impact a variety of other species and ecological processes in the salt marsh.

We conclude that the *Deepwater Horizon* oil spill had substantial impacts on periwinkles in heavily oiled marshes, including density reductions and shifts in size structure, both spanning multiple years. Impacts were ongoing and full recovery had not occurred as of 2015, more than 5 yr post-spill. Our findings support the idea that full periwinkle recovery is likely to be a long-term process, and suggest that monitoring of snail populations should be continued. Finally, oil spill effects on marsh periwinkles are likely to impact a variety of marsh species and processes, and these implications should be considered in future studies.

**Data sources.** NRDA data used in this paper are publicly available at <https://dwhdiver.orr.noaa.gov>. GoMRI data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at <https://data.gulfresearchinitiative.org> (doi: 10.7266/N7FF3Q9S). Data from McCall & Pennings (2012) are publicly available at <http://dx.doi.org/10.6073/pasta/8da296e41363a8fcb931d44a71264107>.

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of the authors and not necessarily of all participants in the cooperative studies upon which the paper is based. We thank NOAA, the State of Louisiana, the National Science Foundation, and the Gulf of Mexico Research Initiative for funding support.

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# Seagrass response following exposure to *Deepwater Horizon* oil in the Chandeleur Islands, Louisiana (USA)

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**ABSTRACT:** The Chandeleur Islands, Louisiana (USA), were among the first coastal locations in the northern Gulf of Mexico (GoM) threatened by exposure to *Deepwater Horizon* oil. Shoreline oiling data and surface oil trajectories (aerial and satellite imagery) showed oil passing through seagrass beds on the shallow back barrier shelf west of the islands repeatedly between May and early July 2010. Aerial photos in May 2010 revealed a heterogeneous distribution of surface oil crossing the shelf, and MC252 exposure was confirmed in sediments and seagrass tissue during field assessments. We observed 5 seagrasses growing at densities comparable to other northern GoM communities. *Ruppia maritima* and *Halodule wrightii* were the most common, followed by *Thalassia testudinum*. *Syringodium filiforme* and *Halophila engelmannii* were rarely encountered. The subtidal and intertidal seascape on the shelf was a mosaic of seagrass patches distributed in varying sizes among unvegetated and sparsely vegetated areas at water depths and in sediment types known to support seagrasses. To quantitatively assess the seagrass response following exposure, sophisticated change detection methodologies were applied to aerial photography acquired in October 2010, 2011, and 2012 in a subsample of 5 locations on the shelf where *Deepwater Horizon* oil exposure was confirmed. The analysis conservatively estimated a seagrass loss of 104.22 acres (42.18 ha) at these locations. Unexpectedly, the whole back barrier shelf experienced a net gain of 228 acres (92.27 ha) of seagrass between 2010 and 2011, representing a pause in the long-standing trend in seagrass declines in the Chandeleurs and indicating that oil exposure did not result in a shelf-wide catastrophe for seagrasses. Predictions for the impending disappearance of this seagrass resource in the near future may need to be reconsidered.

**KEY WORDS:** Oil spill · Gulf of Mexico · Seagrass communities · Change detection

## INTRODUCTION

The shallow coastal waters of the northern Gulf of Mexico (GoM) are ecologically important habitats for fish, shellfish, and wildlife that provide highly valued recreational, economic, and aesthetic services to society. Seagrasses are among several coastal habitats

that contribute to attaining these values by providing a wide range of ecological services (Michot & Chadwick 1994, Beck et al. 2001, Duarte et al. 2005, Larkum et al. 2006, Orth et al. 2006b, Waycott et al. 2009, Barbier et al. 2011). In the northern GoM, 6 seagrass species are widely distributed along the coastlines in lagoons, bays, and barrier islands from

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Texas to the panhandle region of northwest Florida (Green & Short 2003, Handley et al. 2007, Ray et al. 2014). They grow in water depths ranging from the intertidal to 10 m, but water clarity generally limits their growth to  $\leq 2.0$  m in nearshore waters, making them especially vulnerable to anthropogenic disturbances, including degraded environmental quality (Orth et al. 2006b, Waycott et al. 2009) and exposure to oil (Zieman et al. 1984). Seagrasses are considered 'ecological engineers' (van der Heide et al. 2012) because their leaf canopies baffle water flow and wave energy, and belowground rhizome and root structures bind and stabilize substrates (Fonseca et al. 1983, Fonseca & Bell 1998, Duarte & Chiscano 1999). These attributes promote the deposition and retention of sediments, organic matter, and other materials within the meadows and are widely cited as some of the major functions of seagrass beds (Larkum et al. 2006). However, these important functions may also be an 'Achilles heel' in an oil spill. In shallow water and intertidal areas, seagrass canopies can extend well up into the water column, sometimes even to the water surface, where they can come into direct contact with oil, weathered petroleum, and dispersants during a spill. At the same time, sea-

grasses can promote the deposition of contaminants associated with suspended sediments and organic matter. Once these contaminated materials are deposited, they are not easily resuspended or transported out of the meadows, and are likely to be retained and concentrated in the leaf canopy and sediments where important bio-physical processes can become exposed to toxic chemicals.

Following the *Deepwater Horizon* (DWH) explosion on 20 April 2010, Macondo oil from the Mississippi Canyon Block 252 (MC252) traveled upward from the wellhead through the 1.5 km water column to form expansive surface oil slicks which were transported to nearshore coastal ecosystems by wind and currents (Peterson et al. 2012). Synthetic Aperture Radar (SAR) detection of surface oil (Graettinger et al. 2015) and shoreline surveys (Michel et al. 2013) demonstrated light to heavy oiling events in nearshore environments as far west as Atchafalaya Bay, Louisiana (LA), and as far east as Apalachicola, Florida (FL) (Fig. 1). Some shorelines were repeatedly oiled during the spill and long after the wellhead was capped, as early as 3 May 2010 (Michel et al. 2013) and as late as 26 July 2010 (<http://gomex.erma.noaa.gov/erma.html>; Daily Integrated Oil Cover).

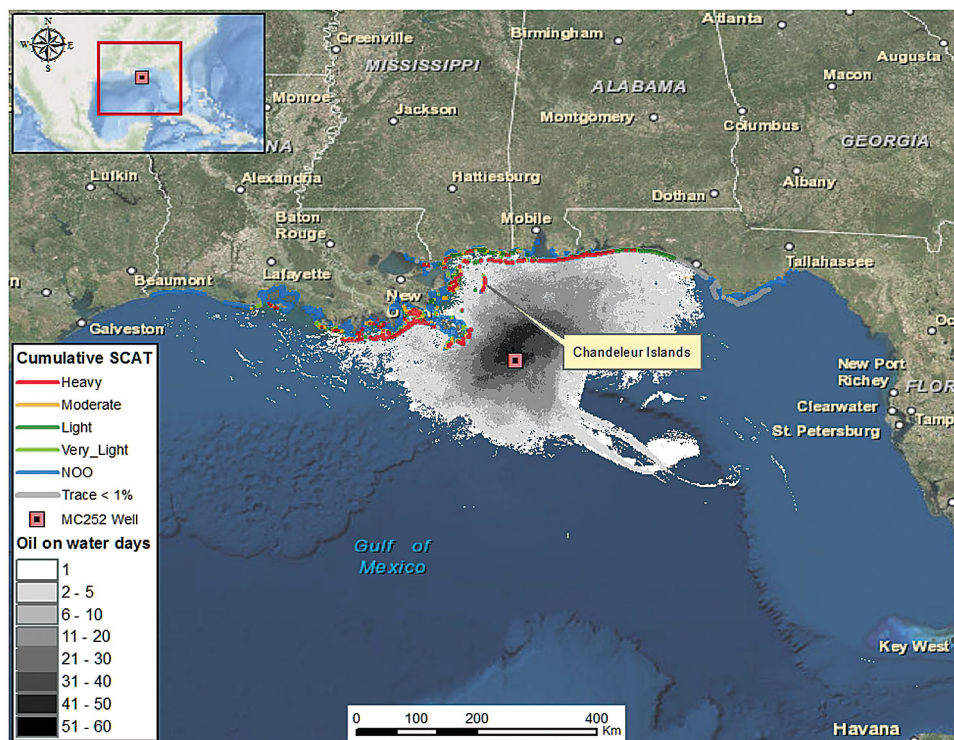


Fig. 1. Location of the Chandeleur Islands, Louisiana, USA, in the northern Gulf of Mexico ( $29.84^{\circ}$  N,  $88.84^{\circ}$  W), showing the estimated number of days that oil (MC252) from the *Deepwater Horizon* spill remained on the water surface (based on Synthetic Aperture Radar satellite imagery) and extent of cumulative shoreline oiling (assessed by the Shoreline Cleanup Assessment Technique, SCAT). NOO: no oil



In early May 2010, oil began reaching ecologically sensitive shallow coastal habitats in the northern GoM known to have seagrasses, including the offshore barrier islands of LA (Handley et al. 2007) (Fig. 1).

The Chandeleur Islands, LA, were among the earliest locations where seagrass meadows were oiled. Evidence based on direct observations of shoreline oiling by the Shoreline Cleanup Assessment Technique (SCAT) in early May 2010 (Michel et al. 2013) confirmed oiling along a large portion of the western shoreline and fringing marshes of the Chandeleurs (Fig. 2). Oiling of the Chandeleur Islands was confirmed by SCAT surveys as early as 8 May 2010, and oil on water was clearly visible in imagery from the National Agriculture Imagery Program (NAIP) from 10 May 2010 (Fig. 3). Light to heavy oiling was

identified throughout the islands during SCAT surveys conducted from 21 through 28 June and when SCAT teams returned to the Islands from 11 through 13 July (see SCAT survey data from <http://response.restoration.noaa.gov/erma/> - 27 September 2014 Cumulative Oiling Ground Observations).

Several direct impacts of oil and dispersants on seagrasses have been documented, ranging from complete mortality (Foster et al. 1971a,b, Zieman et al. 1984, Thorhaug & Marcus 1987, Jackson et al. 1989, Sandulli et al. 1998, Scarlett et al. 2005) to leaf exfoliation, sublethal stress and chronic impairment of seagrass and sediment metabolism and function (Hatcher & Larkum 1982, Ralph & Burchett 1998, Peirano et al. 2005) (see Supplement 1 at [www.int-res.com/articles/suppl/m576p145\\_supp.pdf](http://www.int-res.com/articles/suppl/m576p145_supp.pdf)). Sec-

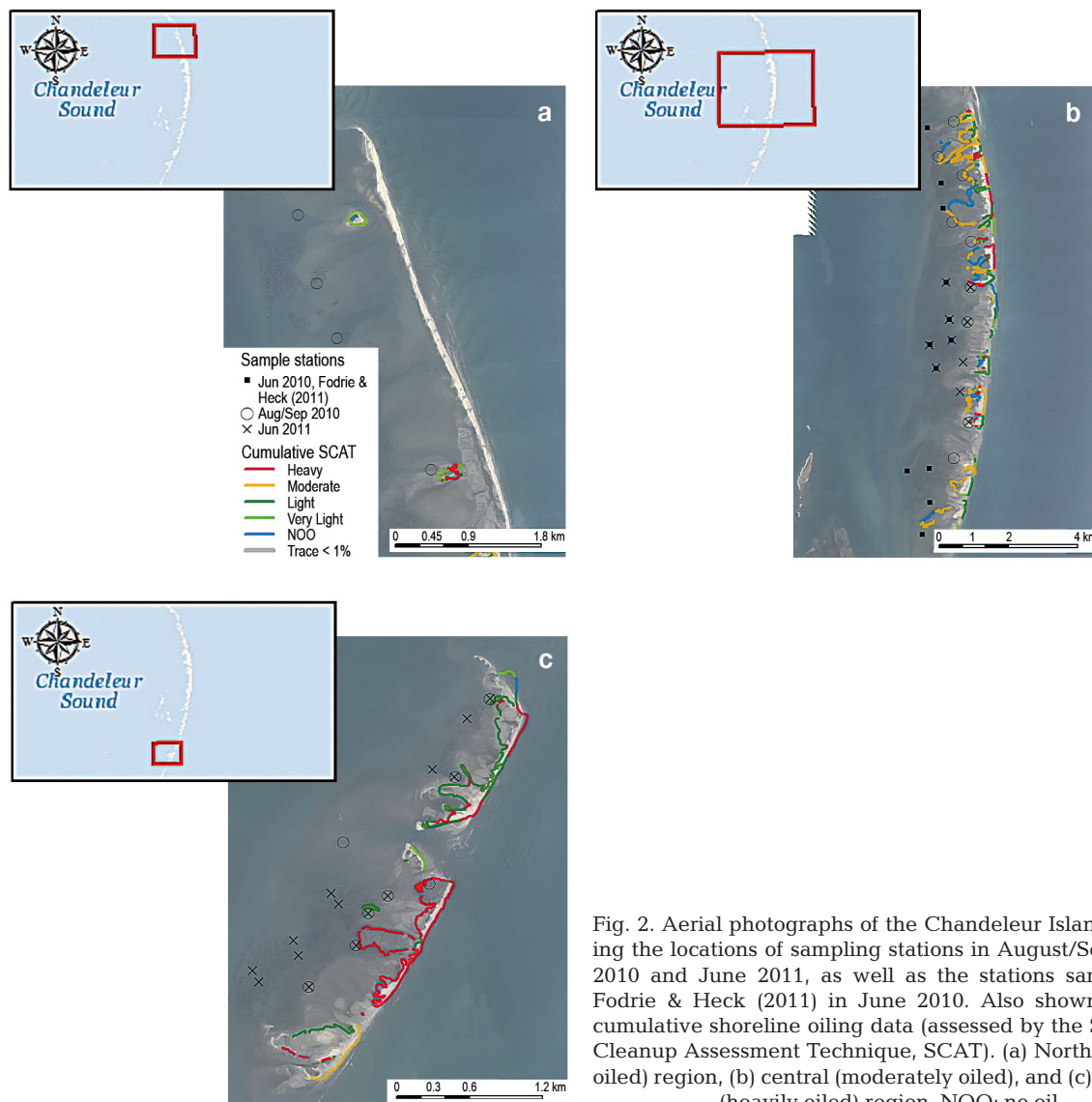


Fig. 2. Aerial photographs of the Chandeleur Islands showing the locations of sampling stations in August/September 2010 and June 2011, as well as the stations sampled by Fodrie & Heck (2011) in June 2010. Also shown are the cumulative shoreline oiling data (assessed by the Shoreline Cleanup Assessment Technique, SCAT). (a) Northern (non-oiled) region, (b) central (moderately oiled), and (c) southern (heavily oiled) region. NOO: no oil

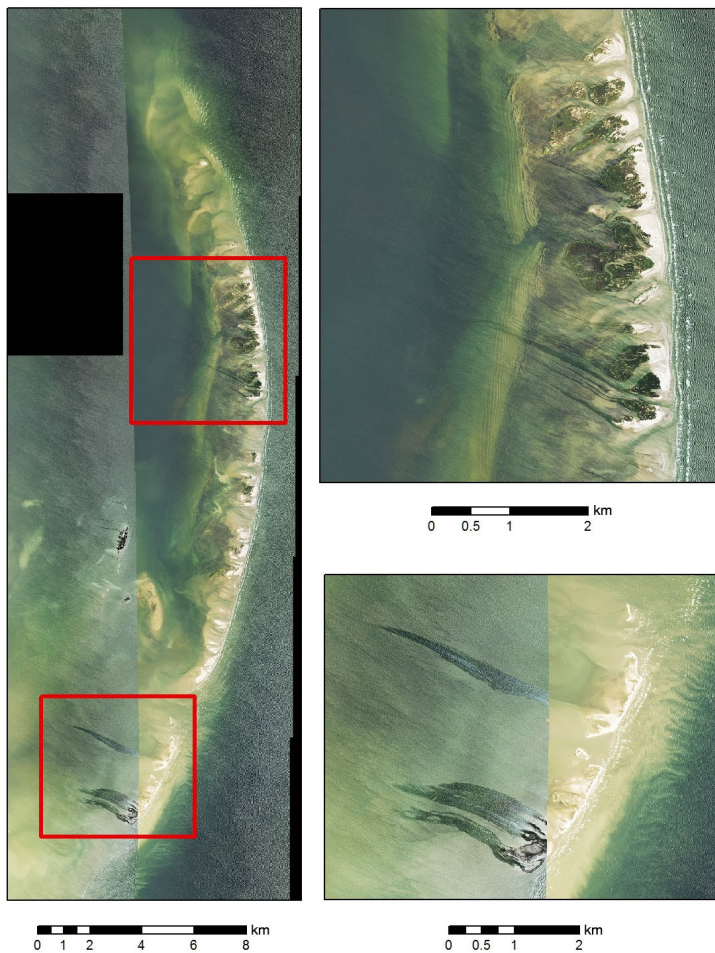


Fig. 3. National Agriculture Inventory Program (NAIP) aerial photographs taken in May 2010 showing surface sheens of oil on water in the central and southern regions of the Chandeleur Islands. Areas in the red squares are enlarged in the panels to the right

ondary impacts also include biophysical and chemical disturbance to sediments, microbes, microfauna, and microflora, epiphytes, and the impairment and mortality of secondary producers residing in the seagrass canopy and sediments (e.g. invertebrates, crustaceans, fishes, waterfowl) (Diaz-Piferrer 1962, Tatem et al. 1978, den Hartog & Jacobs 1980, Carls & Meador 2009). Response actions and cleanup efforts in seagrass beds and adjacent shorelines can also cause environmental degradation and seagrass loss (Zieman et al. 1984, NOAA 2011). In several field assessments following oil spills, the most severe impacts to seagrasses were documented in shallow subtidal and intertidal habitats where petroleum and weathered byproducts came into prolonged direct contact with seagrasses, fauna, other flora, and sediments. Generally, there is agreement that deeper subtidal meadows are less vulnerable to exposure (Zie-

man et al. 1984, Durako et al. 1993), and the potential impacts of oil depend on the unique bio-physical characteristics of a site, such as distance from the source, weathering, tidal range, water depth, wind and wave exposure, currents, substrate type, and species composition (Zieman et al. 1984, Kenworthy et al. 1993, Hayes et al. 1993).

Given the wide range of potential impacts of oiling on seagrasses and the documentation of *Deepwater Horizon* oiling in shallow waters and shorelines of the Chandeleurs, the objectives of this study were (1) to determine the degree and extent of *Deepwater Horizon* oil exposure in seagrass meadows at the Chandeleur Islands, (2) characterize the communities of seagrasses exposed, and (3) quantitatively assess the distribution and abundance of seagrasses following the exposure.

## MATERIALS AND METHODS

### Study site

The Chandeleurs are a north–south oriented chain of narrow, arcuate, transgressive barrier islands slightly elevated above sea level and located ~50 km south of Gulfport, Mississippi, and 125 km north of the *Deepwater Horizon* oil well (Fig. 1). Seagrass meadows occur on a shallow shelf extending 1 to 2 km westward of the islands to a slightly elevated subtidal back barrier bar where the shelf terminates in the relatively deeper water of Chandeleur Sound (Poirrier & Handley 2007, Pham et al. 2014). The islands deflect wind, wave, and current energy from the open GoM, making the western fringing shelf a semi-protected lagoon-like environment with shallow water ( $\leq 2.0$  m) well within the photic zone and physically ideal for seagrasses to thrive.

### General sampling design and chronology

#### Initial exposure sampling (June 2010)

Sediment and plant samples were collected opportunistically in conjunction with an ongoing project designed to sample 12 locations with seagrasses in the Chandeleur Islands (Fodrie & Heck 2011; Fig. 2).

Despite initial efforts to conduct pre-spill sampling of ambient conditions, we were unable to acquire samples at the Chandeleur Islands until 2 June 2010; therefore, pre-exposure ambient concentrations of oil-related compounds at the Chandeleur Islands could not be confirmed.

#### Post-spill exposure sampling (August–September 2010)

Initially, the SCAT data were used to qualitatively establish a preliminary stratification of the Chandeleur Islands into (1) heavy oiling (southern region), (2) moderate oiling (central region), and (3) no oiling (northern region) and were incorporated into field sampling plans between 31 August and 2 September 2010 (Fig. 2). The sampling plan relied on the best available knowledge of seagrass presence/absence based on prior mapping studies (Handley et al. 2007) and knowledge of the proximity of known seagrass beds to oiled shorelines determined by SCAT surveys. For initial selection of sampling stations, post-Hurricane Katrina seagrass survey data from the coast of Mississippi and Alabama (Byron & Heck 2006) were used to help determine sampling density for small, discontinuous submerged aquatic vegetation (SAV) beds in the northern GoM. Statistical analysis of these data determined that a distance of 400 m between sample stations was required to meet the following decision parameters: Type I error or  $\alpha$  (erroneously declaring a site non-impacted) = 5% (significance or confidence = 95%); Type II error or  $\beta$  (erroneously declaring a site impacted) = 10%, or a test power of 90% to correctly reject the null hypothesis; and test resolution or  $\Delta$ , which is the percent change in mean value detected at 95% confidence. Additionally, a target distance of approximately 500 m between stations was determined sufficient to detect the desired percent change in observed SAV conditions at a site relative to those observed at a comparable reference site with 95% confidence (Type I error = 5%) and 90% statistical test power (Type II error = 10%).

#### Post-spill exposure sampling (June 2011)

Based on further inspection of SCAT data, surface oiling in NAIP aerial imagery, and laboratory screening of the initial exposure samples obtained in 2010, the preliminary stratification of oiling categories were modified in June 2011 to include only the cen-

tral and southern regions of the Chandeleurs with the addition of more sampling stations to gain wider spatial coverage across the shelf (Fig. 2b,c). Several stations from 2010 were also resampled to determine persistence of exposure over time.

### Oil exposure sampling methods and analyses

#### Sediment samples

Samples were collected in the top 2 cm of the sediment and subjected to laboratory analysis for total polycyclic aromatic hydrocarbon (tPAH) surrogate-corrected concentrations, calculated based on the summation of 54 PAH analytes including parent PAHs and selected alkylated homologs listed in Forth et al. (2015) (see Supplement 2 at [www.int-res.com/articles/suppl/m576p145\\_supp.pdf](http://www.int-res.com/articles/suppl/m576p145_supp.pdf) for a list of PAHs). If the concentration of a given compound in a sample was not detected, it was treated as a 0 value in the summation. Two other PAH sums were also used to help distinguish types of PAHs: (1) PAHs associated with petroleum, like oil from the DWH spill (petrogenic) and (2) PAHs associated with the partial combustion or pyrolysis of organic matter (pyrogenic) (Emsbo-Mattingly & Martin 2015).

PAHs, including alkyl homologues, were analyzed in compliance with modified US Environmental Protection Agency method 8270 (US EPA 2015), utilizing gas chromatography with low-resolution mass spectrometry in the selected ion monitoring mode. The analytical chemistry results were subjected to rigorous quality assurance/quality control and validation procedures, as described in the NRDA Analytical Quality Assurance Plan (NOAA 2014) and Data Validation Plan (EcoChem 2011), respectively.

#### Seagrass tissue

Plant tissue samples identified as leaves and stems were obtained from the seagrass meadow canopy and sub-sampled twice in the laboratory. One sub-sample, referred to as 'external materials,' included only epiphytes and inorganic material loosely adhered to the leaves. This material was gently washed from the leaves and rinsed with dichloromethane, and the resulting rinsate was dried and measured for PAHs. The other sub-sample was rinsed with deionized water and homogenized before analysis. PAHs were analyzed as described above for the sediment samples.



### Confirmation of oiling using SAR data

SAR identifies very thin surface oil slicks by detecting a change in backscatter resulting from oil-dampening surface capillary waves (Graettinger et al. 2015). SAR data were collected, compiled, and analyzed from 25 April to 26 July 2010, and an estimate of cumulative oiling per 50 m × 50 m grid area was calculated by adding overlapping grid cells to determine total oiled days. In order to calculate the average oil on water days for a particular site, seagrass polygonal areas delineated using fall 2010 aerial imagery (see Fig. 5) were spatially joined to the cumulative oiling raster grid dataset in ArcGIS software (Fig. 4). Each seagrass area less than 2500 m<sup>2</sup> was associated with the closest 2500 m<sup>2</sup> grid estimate, while larger seagrass areas were assigned the average oil on water days for encompassed grid cells. Finally, a spatially-weighted average number of oil on water days was calculated for the Chandeleur Islands.

### Characterization of Chandeleur Island seagrasses

Post-exposure sampling (August/September 2010)

Initially, the selection of sample sites was guided by the criteria described above. Seagrass characteri-

zation and exposure sampling were conducted at the same stations: (1) no oiling in the northern region (n = 4), (2) moderate oiling in the central region (n = 9), and (3) heavy oiling in the southern region (n = 8), for a total of 21 stations (Fig. 2).

Field assessments of the cover, abundance, and general condition of seagrass beds in the Chandeleur Islands were conducted between 21 August and 2 September 2010, almost 4 mo after initial oiling. Field teams navigated to pre-determined geographic coordinates and verified the presence or absence of seagrass. If seagrass was absent, snorkelers conducted systematic circle searches until seagrass was located, at which time the alternate waypoint was stored in the GPS as the final sampling location.

The species composition and percent cover of seagrass at each station was estimated using a modified Braun-Blanquet visual assessment technique averaged from 0.25 m<sup>2</sup> PVC quadrats (n = 3) haphazardly tossed into the seagrass bed (Braun-Blanquet 1932, Kenworthy et al. 1993, Fourqurean et al. 2001). Additionally, 3 plant/sediment cores (15 cm diameter) were collected to estimate shoot density of individual species. During all sampling, observers noted if oil was present on the seagrass or sediments and made qualitative observations of oiling and the health and condition of leaves, whole shoots, rhizomes, roots, apical meristems, new shoot and rhizome growth, and epiphytes.

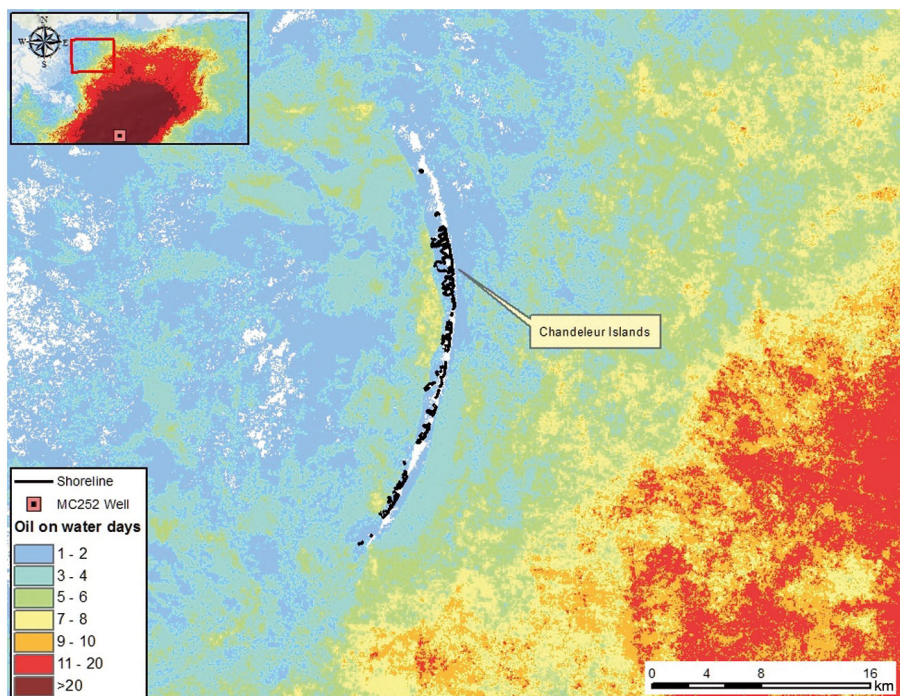


Fig. 4. Estimated number of days that oil from the *Deepwater Horizon* spill remained on the water surface at the Chandeleur Islands (based on Synthetic Aperture Radar satellite imagery)

### Post-exposure sampling (June 2011)

Field assessments and sample processing (20 to 22 June 2011) followed the same general protocols as in September 2010 with 3 exceptions: (1) the original oiling stratification categories were modified to focus only on the central region (formerly referred to as moderately oiled;  $n = 10$ ) and southern region (formerly referred to as heavily oiled;  $n = 14$ ; Fig. 2b,c); (2) sampling intensity was increased to 5 quadrats and 5 cores within each stratum; and (3) inshore, mid-shore, and offshore stations were sampled to gain more comprehensive shelf-wide information about the seagrass beds.

### Aerial imagery interpretation, seagrass mapping, and change analysis

A hybrid imagery analysis technique incorporating automated object-based imagery analysis (OBIA) (Benz et al. 2004) and traditional photo-interpretation methods (Handley et al. 2007) was used to map the presence/absence of seagrasses in high-resolution aerial imagery acquired on 3 dates in the fall of 2010, 2011, and 2012 (Fig. 5) (Handley et al. 2010, NOAA 2013). Specific additional details on the hybrid method are provided in Supplement 3 at [www.int-res.com/articles/suppl/m576p145\\_supp.pdf](http://www.int-res.com/articles/suppl/m576p145_supp.pdf). Fall imagery was the first available opportunity and coinci-

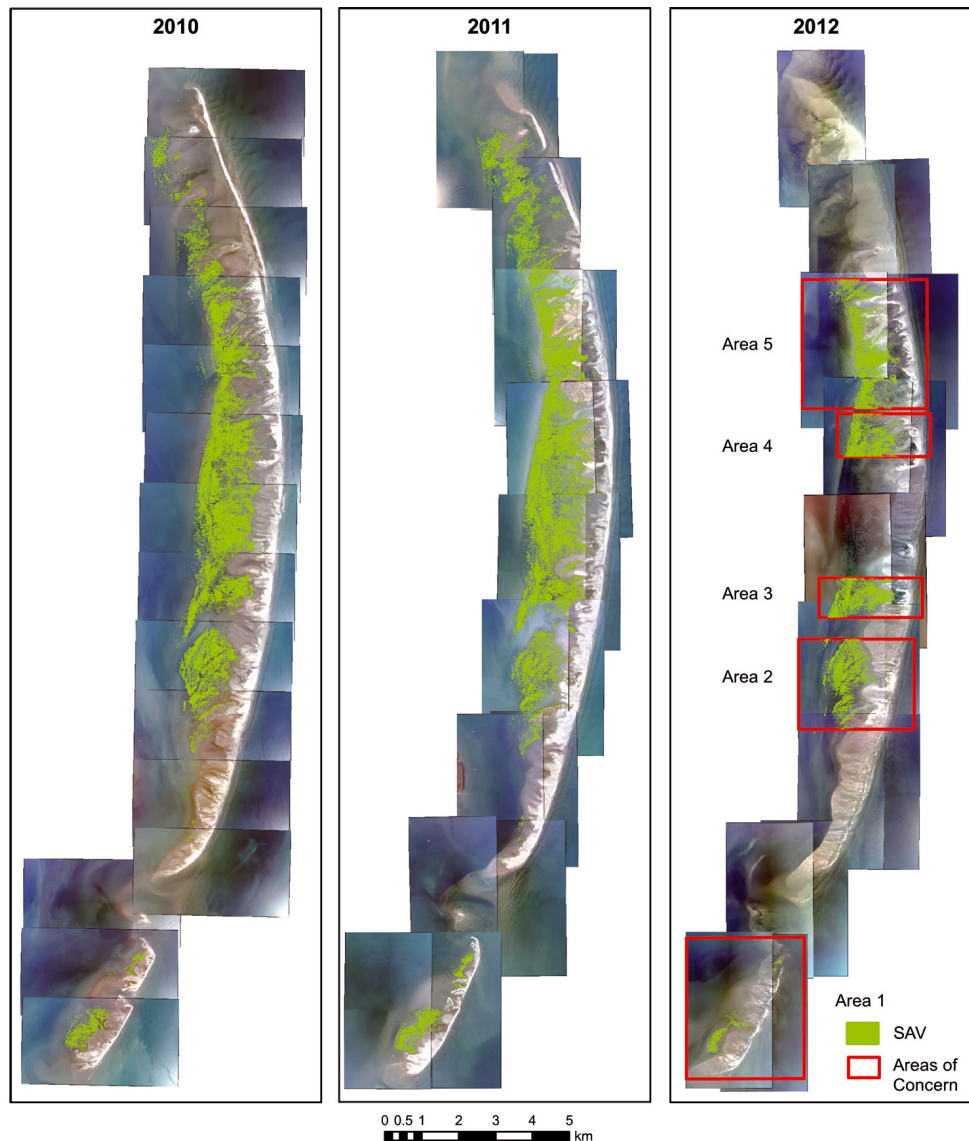


Fig. 5. High-resolution aerial photograph and interpreted classification of seagrass (submerged aquatic vegetation, SAV) presence (green areas) for 3 years (October 2010, 2011 and 2012). Also shown highlighted in the red polygons are the 5 areas of concern where detailed change analyses were conducted



dently the preferred signature index period, since the meadows were expected to be at their maximum extent and biomass. There was no reliable source of pre-spill imagery, so October 2010 imagery was considered baseline for the analysis. The 12-bit imagery, with a pixel resolution of 0.3 m, was collected using multiple DMS sensors and met ASPRS Class 2, 1:24 000 scale accuracy standards, which limit the RMS error in *x* or *y* to 1.2 m. Complementary ground truth information on seagrass distribution, abundance, and species composition were obtained during field sampling at the Chandeleur Islands on 2 June 2010 (Fodrie & Heck 2011), between 31 August and 2 September 2010, and again between 20 and 22 June 2011.

The mapping and change analysis classification scheme was binary (presence/absence of seagrass), consisting of 2 classes: seagrass (SAV) and not seagrass (NOT SAV). Although the classification scheme was binary, samples of many other features were identified in order to be removed from consideration as seagrass, and these consisted primarily of sand substrates and other non-seagrass features (e.g. detritus). The change detection analysis consisted of 2 parts: (1) change between 2010 and 2011 along the full length of the Chandeleur Islands, and (2) change between all 3 dates of mapping (2010, 2011, and 2012) within 5 areas of concern (AOCs) identified within the Chandeleur Islands based on plumes of surface oil observed in NAIP imagery (10 May 2010; Fig. 3) in combination with SCAT information (Fig. 2) and field data indicating exposure to MC252 oil (see 'Results').

Since the objective of the change detection was to identify areas of seagrass loss corresponding with documented oil exposure, we refined the analysis to discriminate between natural losses and those potentially related to oil exposure. 'Core areas' were identified and delineated within each of the AOCs (see Supplements 3 & 4 at [www.int-res.com/articles/suppl/m576p145\\_supp.pdf](http://www.int-res.com/articles/suppl/m576p145_supp.pdf)). Core areas represent areas where imagery interpretation could not be attributed to seagrass losses due to natural processes between 2010 and 2012.

## RESULTS

### tPAH exposure

#### Sediments

The highest tPAH concentrations,  $312 \pm 184$  ppb (mean  $\pm$  SE), were recorded during sampling between 31 August and 2 September 2010 when values were nearly 8 times higher than initially observed on 2 June 2010 ( $40.8 \pm 9.1$  ppb; Table 1). Likewise, the maximum recorded value in the August/September samples was also demonstrably higher (3998 ppb) than the maximum in June (105 ppb). Concentrations of petrogenic PAHs followed the same temporal pattern, with higher values in August/September ( $285 \pm 171$ ; max = 3713 ppb) than first recorded in June ( $27.0 \pm 6.1$  ppb; Max = 75.4 ppb) (Table 1). In June 2011, approximately 1 yr after initial oiling occurred, sediment tPAHs ( $69.7 \pm 9.9$  ppb) and petrogenic PAHs ( $50.7 \pm 9.1$  ppb) had both declined, but were still higher than values recorded initially in June 2010 (Table 1).

#### Plant tissue

As was the case for sediments, concentrations of tPAHs were higher in August/September 2010 ( $44.2 \pm 12.4$  ppb) than in June 2010 ( $3.5 \pm 0.5$  ppb) (Table 2). Likewise, maximum tPAHs (264 ppb) in August/September 2010 were 35 times higher than in June (7.4 ppb). The petrogenic PAH mean ( $38.0 \pm 11.1$  ppb) was also higher in August/September than recorded in June ( $1.4 \pm 0.3$  ppb) and declined 1 yr later, in June 2011 ( $8.2 \pm 4.5$  ppb).

#### External materials

High concentrations of tPAHs were recorded in the external materials rinsed from the seagrass leaves

Table 1. Summary of mean ( $\pm$ SE) total polycyclic aromatic hydrocarbons (tPAH) and petrogenic (PET) and pyrogenic (PYR) PAH concentrations in sediments at the Chandeleur Islands, Louisiana, on 3 sampling dates (given as mm/dd/yyyy). Also shown are the maximum (max) values for each and the number of samples (N). All results are in parts per billion (ppb)

Sampling date	N	Mean tPAH	tPAH max	Mean PET PAH	PET PAH max	Mean PYR PAH	PYR PET max
06/02/2010	12	$40.8 \pm 9.1$	105	$27.0 \pm 6.1$	75.4	$13.6 \pm 3.1$	30.0
08/31–09/02/2010	22	$312 \pm 184$	3998	$285 \pm 171$	3713	$26.3 \pm 12.8$	283
06/20–06/22/2011	52	$69.7 \pm 9.9$	425	$50.7 \pm 9.1$	395	$19.0 \pm 2.1$	91.9

Table 2. Summary of mean ( $\pm$ SE) total polycyclic aromatic hydrocarbons (tPAH), petrogenic (PET), and pyrogenic (PYR) PAH concentrations in 2 sample types: plant tissue (Plant) and external material rinsed from plant tissue (EM) at the Chandeleur Islands, Louisiana, on 3 sampling dates (given as mm/dd/yyyy). Also shown are the maximum (max) values for each and the number of samples (N). All results are in parts per billion (ppb)

Type	Sampling date	N	Mean tPAH	tPAH max	Mean PET PAH	PET PAH max	Mean PYR PAH	PYR PET max
EM	06/02/2010	12	67239 $\pm$ 42789	535598	28710 $\pm$ 16590	208300	38524 $\pm$ 26339	327298
Plant	06/02/2010	12	3.5 $\pm$ 0.5	7.40	1.4 $\pm$ 0.3	2.91	1.9 $\pm$ 0.3	4.09
EM	08/31–09/02 2010	21	174217 $\pm$ 75929	1661945	139890 $\pm$ 70934	1541760	34224 $\pm$ 8460	148970
Plant	08/31–09/02 2010	21	44.2 $\pm$ 12.4	264	38.0 $\pm$ 11.1	234.2	6.2 $\pm$ 1.4	29.9
EM	06/20–06/22 2011	24	47417 $\pm$ 22827	569786	38532 $\pm$ 22277	526910	10974 $\pm$ 2225	42876
Plant	06/20–06/22 2011	26	10.0 $\pm$ 5.0	132	8.2 $\pm$ 4.5	120	1.9 $\pm$ 0.5	12.1

(Table 2). As was the case for both plant tissue and sediments, the highest concentrations were recorded in the August/September sampling (174 217  $\pm$  75 929 ppb) and were 2.6 times greater than in June 2010 (67 239  $\pm$  42 789 ppb). By June 2011, the tPAH concentration had declined (47 417  $\pm$  22 827 ppb) and was less than the value recorded 1 yr earlier. Maximum tPAH values followed the same temporal pattern as did petrogenic PAHs, which were nearly 5 times higher in August/September 2010 (139 890  $\pm$  70 934 ppb) than in June 2010 (28 710  $\pm$  16 590 ppb) and declined to 8.2  $\pm$  4.5 ppb in June 2011.

### Seagrass distribution, species composition, and abundance

August/September 2010

In late August 2010, approximately 3 mo after *Deep-water Horizon* oil first arrived at the Chandeleur Islands, the spatial distribution of seagrasses on the shallow shelf west of the islands was discontinuous (Fig. 5). The subtidal and intertidal landscape consisted of a mosaic of seagrass patches distributed in varying sizes among areas of unvegetated and sparsely vegetated bottom. Generally, meadows in the vicinity of the central region of the islands were larger and relatively more continuous than the meadows in the northern and southern regions, which were more fragmented.

Five of the 6 species of seagrass known to occur in the northern GoM were observed, but only 3 were actually sampled

in the cores and quadrats (Table 3). *Halodule wrightii* was the most commonly encountered species, followed by *R. maritima*. *Thalassia testudinum* was observed at just 2 stations in the central region. *Syringodium filiforme* and *Halophila engelmannii* were observed in the sampling vicinity, but did not occur in any of the quadrats or cores.

Of the 21 stations sampled, 17 were monospecific and 4 were mixed seagrass species stations with the co-occurrence of *H. wrightii* and *R. maritima*. Mixed species stations were observed in both the central and southern regions. Average seagrass cover (all species combined) was 58  $\pm$  21(SE)% in the southern region, 81  $\pm$  14% in the central region, and 78  $\pm$  9% in the northern region. *H. wrightii* densities ranged from 2000 shoots m<sup>-2</sup> in the southern region to 9100 shoots m<sup>-2</sup> in the central region. *R. maritima* densities ranged from 1670 shoots m<sup>-2</sup> in the southern region to 3000 shoots m<sup>-2</sup> in the central region. No *R. maritima* was observed in the northern region. *T. testudinum* densities were 738 and 909 shoots m<sup>-2</sup> at 2 stations in the central region.

Table 3. Post exposure (August/September 2010) characterization of the seagrass species community composition, water depth, and number of stations sampled with each species in the Chandeleur Islands, Louisiana. The stations were stratified into 3 preliminary oil exposure categories based on shoreline oiling data (assessed by the Shoreline Cleanup Assessment Technique): heavily oiled (southern region), moderately oiled (central region), and no oil (northern region). N = number of stations. Values below each species indicate the number of cores in which the species was present

Sampling location	N	Mean depth (m)	<i>Thalassia testudinum</i>	<i>Halodule wrightii</i>	<i>Syringodium filiforme</i>	<i>Ruppia maritima</i>
Southern	8	0.47	0	7	0	3
Central	9	0.42	2	6	0	3
Northern	4	1.1	0	4	0	0

June 2011

In June 2011, we limited our sampling sites to the central and southern regions of the islands, but obtained a wider spatial scope of samples that included inshore, mid-shelf, and outer shelf stations (Fig. 2b,c). As was the case in our first survey, the spatial distribution of seagrasses on the shelf was a mosaic of patches distributed in varying sizes among areas of unvegetated bottom. Four seagrass species were observed and quantitatively documented. The majority (61%) of the cores had just 1 species present, but multispecies meadows were also common (Fig. 6a). At least a third of the stations were comprised of 2-species meadows, consisting of different combinations of *H. wrightii*, *R. maritima*, *T. testudinum*, and *S. filiforme*. Overall, *R. maritima* was the most frequently encountered species, followed in order by *H. wrightii*, *T. testudinum*, and *S. filiforme* (Fig. 6b).

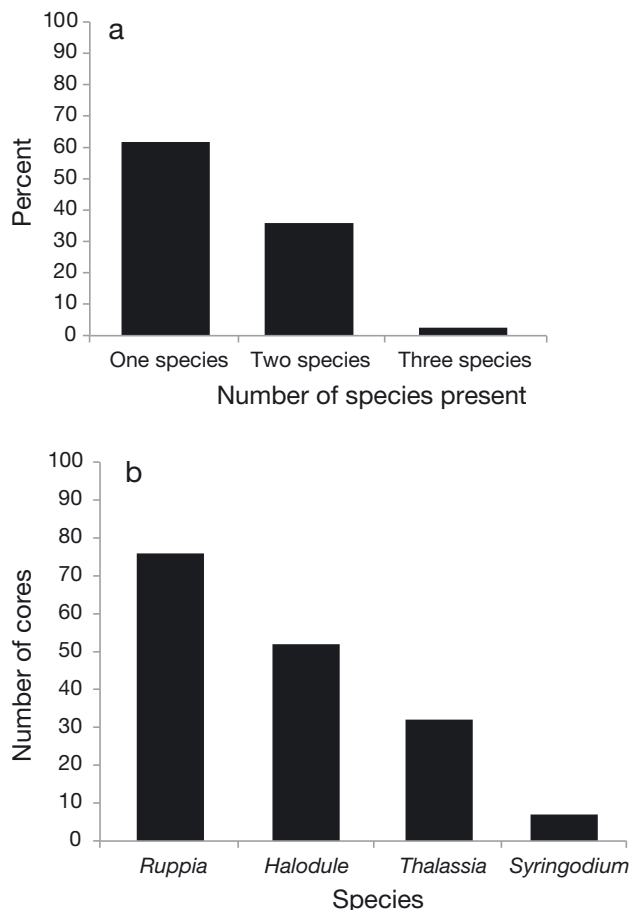


Fig. 6. (a) Percent of total cores having 1, 2, or 3 seagrass species present and (b) number of cores having each individual sea-grass species in the central and southern regions of the Chandeleur Islands in June 2011

There was some variability in the overall species abundance between the 2 regions. In the southern region, *H. wrightii* and *R. maritima* each occurred at 11 of the 14 stations sampled. *H. wrightii* was more frequently observed in the cores than any of the other 3 species (Fig. 7a). *R. maritima* was the next most frequently encountered species. *T. testudinum* was found at 5 of the 14 stations and was the next most frequently encountered species. *S. filiforme* was the least abundant species and was observed at only 1 station. Where seagrasses occurred, total seagrass cover in the southern region ranged from 72 to 100%, averaging  $92.2 \pm 5\%$  and was heavily influenced by a higher abundance of *R. maritima* compared to sampling in August/September 2010.

In the central region of the islands, *R. maritima* was observed at 9 of the 10 stations and was the most frequently encountered species (Fig. 7b). *T. testudinum* was the next most frequently encountered species and was present at 5 of the 10 stations, while *H. wrightii* was less abundant than *T. testudinum* and present at 3 of the 10 stations. As in the southern

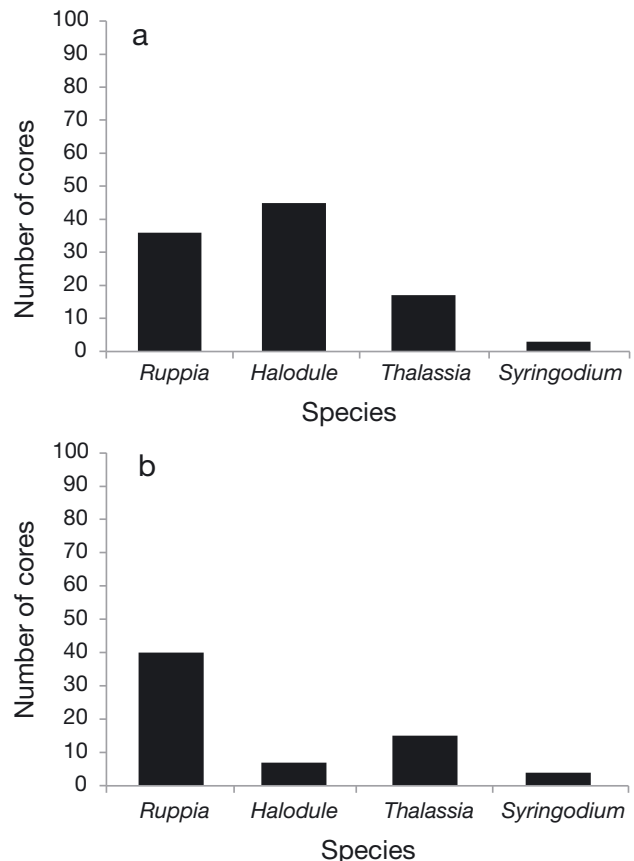


Fig. 7. Frequency of sample cores in which each species occurred in the (a) southern and (b) central regions of the Chandeleur Islands in June 2011

region, *S. filiforme* was the least abundant species. Total seagrass cover in the central region ranged from 45 to 100%, averaging  $90.2 \pm 7.5\%$ .

*R. maritima* densities were lower in the southern region ( $1195 \pm 308$  shoots  $m^{-2}$ ) than in the central region ( $2654 \pm 385$  shoots  $m^{-2}$ ; Fig. 8a). Additional density data obtained in this region in June 2011 ( $1613 \pm 509$  shoots  $m^{-2}$ ; K. Heck unpubl. data) were slightly lower than our samples. Average pre-exposure densities in the central region for *R. maritima* ( $2872 \pm 2159$  shoots  $m^{-2}$ ), obtained between 2006 and 2010 (Fodrie & Heck 2011), were comparable to our June 2011 densities, but had a large standard error, suggesting considerable spatial and/or inter-annual variation.

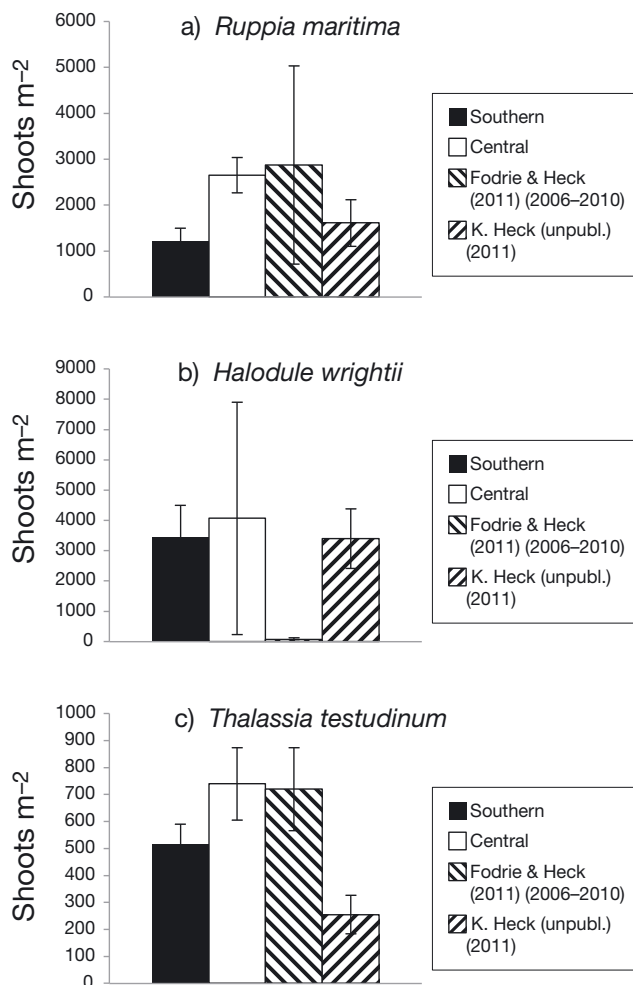


Fig. 8. Shoot density (shoots  $m^{-2}$ ) in the southern and central regions of the Chandeleur Islands in June 2011 for (a) *Ruppia maritima*, (b) *Halodule wrightii*, and (c) *Thalassia testudinum*. Also shown are average shoot densities for the central region between 2006 and 2010 (Fodrie & Heck 2011) and 2011 (K. Heck unpubl. data). Error bars indicate SE

*H. wrightii* densities ranged from  $3451 \pm 1044$  shoots  $m^{-2}$  in the southern region to  $4067 \pm 3840$  shoots  $m^{-2}$  in the central region (Fig. 8b). Additional density data ( $3400 \pm 988$  shoots  $m^{-2}$ ), obtained in the central region in June 2011 (K. Heck unpubl. data), were comparable to our densities. Average pre-exposure *H. wrightii* densities ( $68 \pm 59$  shoots  $m^{-2}$ ) in the central region between 2006 and 2010 (Fodrie & Heck 2011) were much lower than our June 2011 samples (Fig. 8).

*T. testudinum* densities ranged from  $518 \pm 72$  shoots  $m^{-2}$  in the southern region to  $739 \pm 134$  shoots  $m^{-2}$  in the central region (Fig. 8c). Additional density data ( $255 \pm 154$  shoots  $m^{-2}$ ), obtained in the central region in June 2011 (K. Heck unpubl. data), were lower than our densities in June 2011. Average pre-exposure densities in the central region for *T. testudinum* ( $720 \pm 154$  shoots  $m^{-2}$ ) obtained between 2006 and 2010 (Fodrie & Heck 2011) were comparable to our June 2011 samples.

*S. filiforme* was present at only 2 stations, with  $1775$  shoots  $m^{-2}$  in the central region and  $1003$  shoots  $m^{-2}$  in the southern region. These densities were higher than average pre-exposure samples taken between 2006 and 2010 ( $358 \pm 323$  shoots  $m^{-2}$ ; Fodrie & Heck 2011) and samples obtained in the moderate strata in June 2011 ( $738 \pm 237$  shoots  $m^{-2}$ ; K. Heck unpubl. data).

### Seagrass change detection

The first phase of the change detection along the full length of the island between fall 2010 and 2011 detected 483 acres (195.46 ha) of SAV loss and 711 acres (287.73 ha) gained, for a net positive change of 228 acres (92.27 ha). The second phase identified changes between 2010, 2011, and 2012 in the 5 AOCs (Table 4; also see Supplement 2).

Core areas were delineated to omit areas of natural processes that might be responsible for losses. For ex-

Table 4. Summary of changes in seagrass acreage in the 5 core areas in each change class category

Core area	No change	Gain	Loss	Net loss	Net gain
1	46.35	11.0	54.9	43.9	0
2	208.26	35.87	64.44	28.57	0
3	178.28	38.98	36.61	0	2.37
4	178.33	20.99	47.54	26.55	0
5	316.28	60.13	67.7	7.57	0
Total		166.97	271.19	106.59	2.37

ample, the core area within AOC 1 eliminates the overwash fans that were visible in the underlying 2012 post-Hurricane Isaac imagery. These fans resulted when waters washed over the island during the hurricane, depositing fans of sediment on the western side of the island and burying existing seagrass. The northern half of AOC 1 was also excluded, because this part of the island offered little physical protection to erosion and overwash burial, and the seagrass beds in this area had been experiencing serious losses prior to 2010. Subsequently, all 5 AOCs were analyzed to omit areas of natural loss (Supplement 2).

Despite evidence of oil exposure and both persistent and delayed losses, all of the core areas had some seagrass coverage that displayed no change or gains (see Supplement 4). Four of the 5 core areas displayed a net loss, while only 1 (core area 3) displayed a small net gain (2.37 acres, 0.959 ha). A total of 111.7 acres (45.20 ha) were identified as persistent loss and 159.5 acres (64.55 ha) were classified as delayed loss (see Supplement 4). Therefore the total loss (persistent + delayed loss) for all 5 core areas was estimated to be 271.19 acres (109.75 ha; Supplement 4). Accounting for the gains (2.37 acres, 0.959 ha), overall there was a net loss of 104.22 acres (42.18 ha) in the 5 core areas.

## DISCUSSION

### *Deepwater Horizon* oil exposure

The spatial and temporal signature of surface oil in aerial photography and SAR data clearly indicated that the spill threatened seagrass meadows located at the Chandeleur Islands, LA. Forensic analyses confirmed that some of the *Deepwater Horizon* oil was transported in surface waters shoreward into shallow coastal regions throughout the northern GoM, where it was dispersed as floating oil slicks, sheens, emulsions, submerged mats, tar balls, or dissolved in water in the Chandeleur Islands (Stout et al. 2016). Five lines of evidence presented in this study, viz. (1) shoreline oiling (SCAT data), (2) sediment and plant PAH data on 2 sampling dates, (3) concurrent forensic confirmation of *Deepwater Horizon* oil exposure (Stout et al. 2016), (4) visual inspection of oil on water in aerial photography, and (5) estimated oil on water days based on satellite imagery (SAR data), confirmed the exposure of seagrass meadows in the Chandeleurs to *Deepwater Horizon* oil.

The shoreline SCAT data confirmed what was evident from aerial photography; oil had to pass over

and through the shallow shelf and seagrass meadows west of the islands to reach the shoreline. Sediments and plant tissues showed increasing concentrations of tPAHs and petrogenic PAHs forensically matched with *Deepwater Horizon* oil between early June and September 2010 corresponding with repeated oiling events that took place over the 87 d between April and July before the *Deepwater Horizon* well was capped. By June 2011, approximately 1 yr after exposure commenced, forensic analysis continued to detect *Deepwater Horizon* oil, but tPAH and petrogenic PAH concentrations had declined markedly to values only slightly higher than initially recorded in June 2010, indicating that persistent exposure was mitigated by further redistribution, weathering, biogeochemical processing, and degradation of the oil.

### Exposure scenario and seagrass response

The potential susceptibility of the Chandeleur Islands' seagrasses to *Deepwater Horizon* oil exposure is attributed to several factors. These seagrass meadows were only 125 km from the *Deepwater Horizon* source, and because of their north-south orientation, the islands provided minimal physical protection from the oil, which had a direct pathway into the seagrass meadows on the shallow back barrier shelf (Supplement 5 at [www.int-res.com/articles/suppl/m576p145\\_supp.pdf](http://www.int-res.com/articles/suppl/m576p145_supp.pdf)). As oil was transported onto the shelf and ashore from the south and west, the islands acted as a barrier preventing the easterly transport of oil off the shelf. With a relatively small amount of tidal and wind energy to physically transport it away, it is likely that oil was temporarily concentrated on the shallow shelf and contacted the seagrasses directly in soluble and insoluble forms and became adsorbed onto suspended materials (sediments, detritus) and biota (epiphytes) that may have settled to the bottom and further exposed the seagrass and sediments to potentially toxic concentrations of petrochemicals (Supplement 1). The very high concentrations of *Deepwater Horizon* PAHs and petrogenic PAHs in the external materials loosely adhered to the leaves (epiphytes) suggest that this exposure could have affected the epiphyte community, thereby impacting a productive and important trophic component of the system.

Additionally, tar balls were commonly encountered during sampling, and oil that was stranded along the shoreline and in shallow subtidal sediments (Stout et al. 2016, Rouhani et al. 2017, this Theme Section) may have been redistributed back onto the inter-



tidal and subtidal shelf by wave turbulence and tidal energy leading to prolonged exposure. Subtidal, shoreline, and fringing marsh sediments and particulate detritus exposed to oiling are less cohesive than unoiled material, which may have provided a mechanism for repeated oiling of the seagrass meadows long after the well was capped (Silliman et al. 2012, Martin et al. 2015). Additionally, oiled sediments are not nearly as cohesive, and if the roots and rhizomes were impaired by exposure, seagrasses may have been more easily uprooted. This may be a partial explanation of the unvegetated gaps in substrates and water depths that normally would support seagrass.

Two field assessments and 3 consecutive years of high-resolution aerial photography confirmed that the spatial coverage of seagrasses was heterogeneously distributed across the shallow shelf west of the Chandeleur Islands. The shelf seascape was a mosaic of healthy and actively growing seagrasses in varying sized patches and large continuous meadows distributed among unvegetated and sparsely vegetated gaps. Where the seagrasses occurred, the species composition, cover, and density were comparable to seagrass meadows previously described for this region of the northern GoM (Eleuterius 1987, Green & Short 2003, Byron & Heck 2006, Handley et al. 2007, Carter et al. 2011, Fodrie & Heck 2011, Pham et al. 2014). In September 2010, monospecific species communities were more common than multi-species meadows, while in June 2011, multi-species meadows were more frequent with a demonstrably larger presence of *R. maritima*. Some of this variability in species composition can be attributed to differences in the time of sampling, as well as differences in the spatial extent of our 2 sampling efforts. In September 2010, sampling focused almost exclusively on nearshore sites adjacent to the islands, while in June 2011 the survey area expanded to mid-shelf and outer-shelf sites which captured more of the spatial variation in depth, species distribution, and community composition. Based on our 2 surveys, it is evident that some portions of a diverse and structurally complex seagrass community were exposed to MC252 oil for several months between May and August 2010, and some exposure persisted into 2011, while some areas avoided exposure.

In both sampling events, *Ruppia maritima* and *Halodule wrightii* were the most commonly encountered species in high densities throughout the shelf, in both the subtidal and shallow intertidal where leaf canopies reached the water surface and would have been most vulnerable to oil exposure. We also ob-

served *Halodule* and *Ruppia* in nearshore environments adjacent to shorelines with visibly evident accumulations of stranded oil. *Ruppia* frequently co-occurred with *Thalassia testudinum* and was relatively more abundant in the June 2011 samples, which may be indicative of seasonal differences in phenology and growth. *Ruppia* is a euryhaline, relatively fast-growing, opportunistic, and ephemeral species known to flower prolifically and colonize unvegetated areas by seed, while completing its entire life cycle in 4 mo (Moffler & Durako 1987, Dunton 1990, Byron & Heck 2006). *Ruppia* is among the most fecund seagrasses in the northern GoM and relies on rapid vegetative growth, flowering, and seed production in late spring and early summer to replenish biomass and populations. A recent experimental study demonstrated that high sediment oil concentrations impaired the production of inflorescences, fruiting bodies, and root growth of *R. maritima* (Martin et al. 2015). In June 2011, *Ruppia* flowering plants were commonly observed and widely distributed, with canopies occupying the entire water column up to the surface. If the conditions observed in June 2011 represented the conditions in May and June 2010 when oil first reached the Chandeleurs, *Ruppia* would have been especially vulnerable to exposure. The 3 other species also flower and produce seeds, and *H. wrightii* and *Syringodium filiforme* produce seeds that can be buried in sediment seed banks longer than 12 mo before germinating (McMillan 1991, Inglis 1999, Orth et al. 2006a). Flowering and seed production are important for both meadow maintenance and spatial dispersal of seagrasses (Kendrick et al. 2012), and it is likely that sexual reproduction of these species was also affected by *Deepwater Horizon* oil in 2010. Given that oil exposure was detected in sediments for at least 1 yr, it is plausible to conclude that some of the plants and sediment seed banks were chronically exposed, potentially leading to population-level impacts on the seagrasses.

The Chandeleur Islands have experienced a long history of physical instability resulting from severe storms which have reduced the overall physical size and elevation of the islands (Lavoie 2009, Mitchell et al. 2011). These storm effects and changes in water quality, mainly salinity and turbidity, have corresponded with the diminished areal abundance of seagrasses in the region (Eleuterius 1987, Poirrier & Franze 2001, Franze 2002, Poirrier & Handley 2007, Lavoie 2009, Pham et al. 2014). Unexpectedly, our change analysis identified a shelf-wide net gain of 228 acres (92.27 ha) of seagrass coverage between

fall 2010 and fall 2011. This gain is notable because it signals a pause in the long-standing persistent downward trend in total seagrass coverage in the Chandeleurs and suggests that predictions for the demise of this resource may need re-evaluation. These results, combined with the high density, cover, and observed biodiversity, provide reasonable assurance that there has been no widespread catastrophic loss of seagrasses associated with oil exposure in the Chandeleur Islands.

There was, however, a conspicuous widespread distribution of unvegetated and sparsely vegetated gaps in the cover of seagrasses at water depths and in sediment types known to support their growth. Gaps among healthy seagrass meadows are not uncommon (e.g. caused by bioturbation, grazing, or physical disturbance; Uhrin et al. 2011). However, some of the gaps we observed in September 2010 and again in June 2011 corresponded with areas of documented oil exposure, and the persistence or expansion of these gaps and the formation of new gaps were considered indications of seagrass response to exposure. We also suspected that the 3 mo delay in sampling after initial oiling prevented us from directly observing and documenting visible oil entrained and accumulated in the seagrass beds, the highest concentrations of oil the seagrass and sediments may have experienced, and the most acute and potentially severe toxic effects of exposure.

The cumulative lines of exposure evidence all indicated that oiling and the degree of exposure were spatially and temporarily heterogeneous. This variability in exposure limited our scope of inferences with point samples alone. We were also handicapped by the absence of quantitative baseline data for seagrasses at the time of exposure, thus eliminating a statistically robust before and after sampling design. To overcome these limitations, we completed our assessment using high-resolution aerial imagery and quantitative change analysis of seagrass areal distribution. Without reliable pre-exposure imagery, October 2010 served as the starting reference point for seagrass coverage and the possible latent effects of exposure that may have lingered in the ecosystem for up to 2 yr. Our change detection method in the AOCs, where we documented exposure, deviated from traditional approaches which interpret areal seagrass coverage to calculate an absolute change in area between 2 time intervals. Often, the factors potentially responsible for the change in coverage are assumed to be homogeneously distributed, in which case the net gain in seagrass areal coverage documented between 2010 and 2011 would suggest

that oiling had no effect on total seagrass abundance in the Chandeleur Islands. However, this would disregard evidence that oil exposure was heterogeneously distributed in space over a period of several months, and also overlooks the fact that seagrasses were heterogeneously distributed in such a way that the specific locations where oil reached the shelf may or may not have directly contacted the plant canopies and sediments. This alternative approach acknowledged that there could be areas of seagrass loss due to exposure and areas of no change or gain that were minimally exposed or not exposed at all. Furthermore, documenting all 3 categories over time (3 yr) acknowledges the potential latent chronic effects of exposure which were not immediately evident in field sampling following the initial exposure.

Accounting for gains, the change analysis in the 5 core areas estimated a net loss of 104.22 acres (42.18 ha). To minimize uncertainty and strengthen inferences regarding exposure, areas where changes in seagrass coverage could be attributed to natural processes and problematic interpretation signatures were excluded from this analysis. Therefore, it is plausible to attribute the net losses to acute and chronic exposure of critical plant physiological processes leading to seagrass mortality, the impairment of seagrass reproduction (sexual and/or asexual) recruitment and recolonization, and the latent effects of oil exposure on sediment biogeochemical processes. Since the imagery used in this analysis was acquired in October 2010, almost 5 mo after oil first reached the Chandeleurs, this estimate did not capture seagrass changes that might have occurred during and immediately after exposure in the late spring and summer of 2010. Thus, these estimates likely represent a conservative assessment of changes due to exposure.

## SUMMARY

In the summer of 2010, the biophysical conditions on the back barrier shelf at the Chandeleur Islands were favorable to oiling from the DWH spill. Multiple lines of evidence confirmed *Deepwater Horizon* oil exposure on shorelines, in seagrass tissue, and in sub-tidal and intertidal sediments on the shelf. Given the known mechanisms linking petroleum toxicity to both lethal and sublethal impairment of seagrass physiology and sediment metabolism, we investigated the seagrass response to *Deepwater Horizon* oil exposure at the Chandeleur Islands. Despite evi-

dence of widespread oiling on the shoreline, a shelf-wide change analysis conducted between October 2010 and 2011 showed a small increase in seagrass acreage. Additional change analyses were spatially constrained to a sub-set of 5 areas where we documented exposure by 3 independent methods (aerial photography, *in situ* tissue and sediment samples, and satellite remote sensing) and accounted for natural variation and interpretation errors. By restricting our analysis to these 5 locations, we conclude that it is plausible that seagrass losses in these 5 study areas could be attributed to MC252 oil exposure. The apparent discrepancy between shelfwide gains and losses in the 5 areas we sub-sampled may be explained by the very nature of the spill that resulted in a complicated oiling scenario with temporally and spatially heterogeneous exposure. The release of oil from the well lasted for 87 d, and it arrived at the Chandeleurs after variable degrees of treatment and weathering, at different times, during different water level and wind conditions, and at different stages in the life histories of the seagrass species.

Having avoided a shelf-wide catastrophic loss of seagrass due to oil exposure, the Chandeleur Islands continue to support a diverse and extensive habitat of mixed-species seagrass communities providing important and economically valuable ecological services, one of which is physical stabilization of the shallow back barrier shelf that forms the submerged foundation which the westerly migrating barrier depends on to maintain its somewhat tenuous elevation above sea level. Sediments accreting on the shelf are trapped and stabilized by the seagrasses which also help preserve the physical integrity of the back barrier marsh by protecting the emergent wetland fringe from wind wave energy. Thus, seagrasses function to delay the predicted transition of the emergent islands to submerged shoals similar to what has occurred farther south in the Chandeleurs (Lavoie 2009). The notable pause in the shelf-wide decline indicates that recent predictions suggesting the impending extirpation of this unique and important seagrass system needs some reconsideration. Data on the spatial distribution, species composition, and abundance of seagrasses provided by this study can be utilized by scientists and resource managers as a quantitative contemporary baseline for (1) designing and implementing future monitoring programs, (2) developing resource management and restoration plans for the islands, and (3) contemplating future actions in response to natural disturbances and/or anthropogenic disasters in the region.

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# Potential impacts of the 2010 *Deepwater Horizon* oil spill on subtidal oysters in the Gulf of Mexico

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**ABSTRACT:** The explosion of the *Deepwater Horizon* (DWH) drilling platform initiated an unprecedented chain of environmental perturbations that threatened sensitive nearshore habitats in the northern Gulf of Mexico in 2010. Here, we examined subtidal oyster reef populations monitored by state resource agencies prior to and after the DWH incident in the spring–summer of 2010. Fishery-independent surveys were conducted in each of the following Gulf States using either diver-collected quadrat samples (Louisiana, Mississippi, Florida) or dredge surveys (Texas) at fixed sites to assess trends in oyster density of recently settled (spat), juvenile to young adult (seed), or adult (market) oysters. Compared to baseline values (average 2006–2009), the densities of spat, seed, and market oysters were extremely low in 2010, with little recovery in 2011 and 2012 in areas within the central portion of the northern Gulf of Mexico (eastern Louisiana–Mississippi). In contrast, densities of all oyster size classes in western Louisiana and Texas (outside the footprint of oil or freshwater release) and juvenile oysters in Apalachicola Bay, Florida, revealed no consistent pattern of change in 2010 compared to baseline levels in 2006–2009. Thus, major declines in oyster populations occurred within the northern Gulf of Mexico in summer 2010, and populations remained low through 2012. The spatial footprint of this decline is largely coincident with the oiling and freshwater diversion response activities associated with the DWH incident, although many potential confounding factors are also considered. Fisheries-independent datasets offer much-needed baseline data that can be used to assess potential impacts from disturbances.

**KEY WORDS:** *Crassostrea virginica* · *Deepwater Horizon* · Eastern oyster · Gulf of Mexico · Oil spill · Perturbation

## INTRODUCTION

The interface of marine and terrestrial biomes represents one of the more productive environments on Earth. Degradation of nearshore marine habitats that occurs within this interface poses a significant threat to the sustainable provision of natural resources from them. The release of pollutants in marine environments continues to represent a major source of degradation in many nearshore systems as a result of both discrete and chronic exposure to contaminants.

In April 2010, the drilling platform *Deepwater Horizon* (DWH) exploded in the north-central Gulf of Mexico (GoM) and resulted in an oil spill that lasted for 87 d and released hundreds of millions of liters of Louisiana sweet crude oil into the GoM (US District Court 2014, 2015). The inshore advection and subsequent deposition of the oil resulted in oiling of many beaches and marshes located from western Louisiana to the Florida Panhandle. In response to the oil spill, several activities were conducted to mitigate the amount of oiling, including applying almost 7 million

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liters of dispersants in offshore waters (OSAT 2010), releasing freshwater from diversion structures east and west of the Mississippi River, and initiating other response and cleanup activities within nearshore environments.

When anthropogenic impacts occur, a primary challenge is quantifying the magnitude of each impact on ecosystem functions and the associated goods and services. Furthermore, perturbations that occur in nearshore waters often negatively impact coastal ecosystem services such as fisheries landings. While informative, fisheries landing data can be challenging to incorporate into population and stock assessment models due to the difficulties of normalizing these data to standardize for differences in effort among years. This limitation has motivated many resource agencies to collect fisheries-independent data using seasonal and annual surveys of commercially and recreationally valuable fishery species.

By 2006, each US state bordering the GoM (hereafter referred to as 'Gulf states') had implemented subtidal oyster abundance surveys using dredge (Texas) or quadrat (Louisiana, Mississippi, Alabama, Florida) sampling efforts. Although oyster sampling methodologies and sampling effort varied across this region and these differences must be considered when interpreting the data, the surveys do provide baseline data on juvenile and adult oyster densities for multiple years prior to the DWH spill. The surveys also span across the coastal margin of the GoM, thereby encompassing regions potentially impacted by and adjacent to the spill and resulting in a design analogous to that of a before-after-control-impact (BACI) study. Other important variables and related covariates may have influenced oyster survival during or after the spill. For instance, each state manages its oyster resources differently, from decisions around public vs. private leasing and shell planting to historic harvesting regulations. The Gulf States also responded differently regarding opening and even relaxing fishing restrictions vs. closing the fishery directly during and right after the spill. Still, the state oyster surveys offer an unprecedented opportunity to explore the potential impacts of this historically large spill and associated response activities, such as the opening of freshwater diversions in 2010 in southeastern Louisiana, on subtidal oyster reef habitat throughout the GoM.

Within most estuaries in the northern GoM and the Atlantic Ocean, oysters form reefs and provide ecosystem services that human societies value and rely upon (Grabowski et al. 2012). For instance, oysters enhance the recruitment and growth of economically

valuable and ecologically important finfish and crustaceans, thereby augmenting the productivity of these species (Coen et al. 1999, Breitburg et al. 2000, Harding & Mann 2001, Peterson et al. 2003, Soniat et al. 2004, Grabowski et al. 2005, Tolley & Volety 2005). Oyster reefs concentrate bottom deposits of feces that promote bacterially mediated denitrification, thereby counteracting anthropogenic nitrogen loading (Newell et al. 2002, Piehler & Smyth 2011, Carmichael et al. 2012, Kellogg et al. 2013, Smyth et al. 2013). When oyster reefs filter the water and enhance light penetration, they promote other valuable estuarine habitats such as submerged aquatic vegetation (Newell 1988, Everett et al. 1995, Newell & Koch 2004, Carroll et al. 2008, Wall et al. 2008). Thus, large-scale perturbations that cause widespread oyster mortality and the degradation of oyster reef habitat also can result in the loss of these valuable ecosystem services. Given that an ecosystem services approach has been suggested as a possible mechanism to address the natural resource damages caused by the DWH spill (National Research Council 2013), quantifying the magnitude of damages to species such as the eastern oyster *Crassostrea virginica* is an important first step.

Here we assessed nearshore subtidal oyster reef populations in the GoM using fisheries-independent data collected before (2006–2009) and after (2010–2012) the DWH spill. We excluded data collected before 2006 in our baseline period because of the impacts to the GoM from hurricanes Katrina and Rita. Moreover, including years prior to 2006 when these major disturbances occurred would potentially confound our efforts to use the baseline period to predict what oyster abundances would have been in 2010–2012 in the absence of the spill. We compared baseline and post-spill estimates of spat-, seed-, and market-sized oysters from Texas to Florida. Thus, we were able to examine potential impacts from the DWH spill and response activities in areas located proximal to the spill (i.e. Louisiana and Mississippi) vs. those that were more distant and not directly impacted by it (i.e. Texas and Florida).

## MATERIALS AND METHODS

### Data collection

Oyster abundance data from 2006–2012 were obtained from the marine resources management agency for each of the Gulf States (Fig. 1). These data were collected using the standard methods histori-



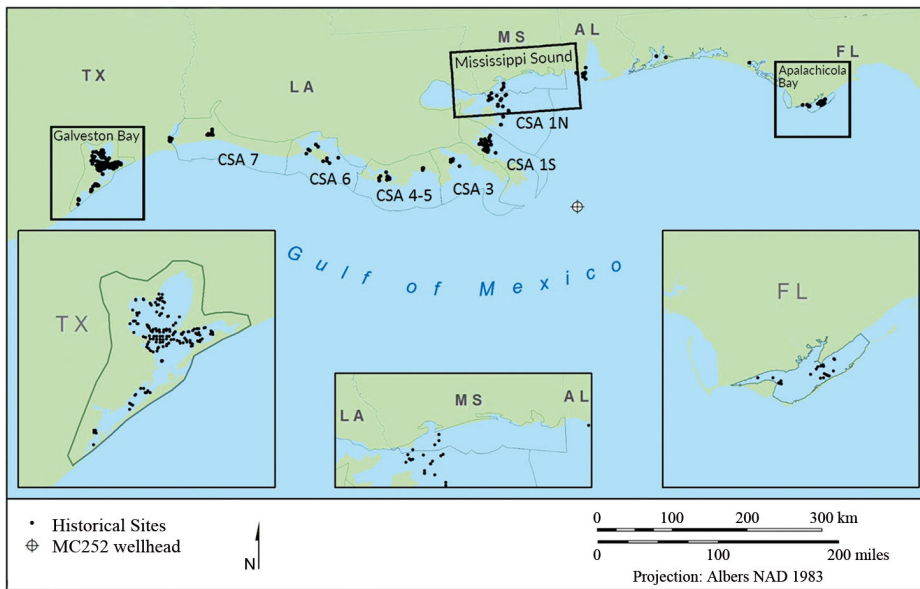


Fig. 1. Location of sampling sites in each state and the MC252 (*Deepwater Horizon*) wellhead. Coastal study areas (CSAs) are depicted in Louisiana (LA). TX: Texas, MS: Mississippi, AL: Alabama, FL: Florida

cally used by each state (see below for greater detail). Data from the state monitoring sites were reviewed to ensure that sites were sampled both in the comparison year (2010, 2011, or 2012) and in at least 2 out of the 4 baseline years (2006–2009). The methods used to collect and measure oyster abundance data varied by state and are described below. In all cases, oyster shell height (SH) was defined as the maximum measurement from the umbo to the ventral margin of the oyster.

#### Texas

We obtained oyster data from annual dredge surveys conducted in Galveston Bay by Texas Parks and Wildlife. Dredge samples were collected monthly in areas that were known to contain oyster reef habitat. Sampling was conducted by towing a dredge at 5 km h<sup>-1</sup> for 30 s behind a boat following the contour of the reef. The dredge was 0.5 m wide × 0.24 m high × 1.0 m long with a 76 mm nylon mesh bag to retain oyster shell material. From the dredge contents, all living oysters >25 mm SH were counted, and 19 of these oysters were randomly selected and measured to the nearest mm (SH). The number of dead oysters in the dredge was also counted. The number of spat oysters (5–25 mm SH) was enumerated on 1 randomly selected side of up to 5 living and 5 dead oysters.

#### Louisiana

The Louisiana Department of Wildlife and Fisheries (LA-DWF) annually estimated oyster abundance at ~100 sites on public oyster grounds each July from 2006 to 2012. Samples were collected using 1.0 m<sup>2</sup> quadrats randomly placed on an oyster reef at each site. A SCUBA diver removed all oysters within the frame to a depth of approximately 7.5–10 cm and brought the sample to the surface where it was processed. The height of all living oysters was measured and recorded in 5 mm increments (e.g. 0–4, 5–9 mm, etc.). At least 2 replicate 1 m<sup>2</sup> samples were conducted at each site.

While LA-DWF conducted oyster surveys of the 1.68 million acres (~680 000 ha) of public bottom available for harvest, an additional ~400 000 acres (~162 000 ha) are under lease. The public oyster grounds are typically used as a source of sublegal oysters that are transplanted to private leases, although they can also account for a substantial proportion of oyster landings in Louisiana (e.g. public grounds accounted for 47% of oyster landings in Louisiana in 2007; LA-DWF 2011). Thus, the public oyster grounds are considered critically important to the Louisiana oyster resource (LA-DWF 2011).

#### Mississippi

In Mississippi, 5 reefs were surveyed annually by the Mississippi Department of Marine Resources at multiple (14–20) randomly selected subplots on the reefs. At each site, a SCUBA diver collected oysters using a 1 m<sup>2</sup> quadrat on each reef. All oysters within the quadrat to a depth of 7.5–10 cm were collected and brought to the surface for processing. SHs of all living oysters were recorded to the nearest 1 mm.

#### Florida

We obtained quadrat data from the Florida Department of Agriculture and Consumer Services Division for Aquaculture for 23 sites in Apalachicola, Pen-

sacola, and St. Andrew's Bays. Transects were established that traversed oyster reefs at each site, and multiple (up to 20) 1 m<sup>2</sup> quadrat samples were selected by throwing the quadrat frame overboard. Divers removed oysters to a depth of approximately 15 cm. SHs of all living oysters were rounded down to the nearest 5 mm. Density estimates per 1 m<sup>2</sup> were then calculated for oysters  $\geq 50$  mm,  $\geq 75$  mm, and overall.

### Data processing

We attempted to compile these data for each state in such a manner that they were as consistent as possible across regions. This effort was limited in instances where the data were collected using a different field method (e.g. dredge data from Texas), or where we received summarized data from the state and could not reclassify the data by our defined size classes (e.g. density data from Florida). Where possible, state oyster abundance data were compiled into 3 categories: spat (generally <25 mm SH), seed (generally 25–74 mm SH), and market ( $\geq 75$  mm SH; Table 1). All abundance metrics for Louisiana, Mississippi, and Florida were converted to the number of living oysters of a given size class per 1 m<sup>2</sup>. These results were used to calculate mean oyster density at each site. Because oyster dredge efficiency can vary with oyster size and reef properties (Powell et al. 2002), oyster data from Texas provide an index of density rather than a quantitative estimate of oyster density.

The following computations were conducted to standardize comparisons among the different regions and periods. In Texas, abundances of seed- and market-sized oysters were calculated as mean abundance site<sup>-1</sup> yr<sup>-1</sup>, scaled by the number of oysters in the sample. The seed and market data had to be scaled to account for the fact that only the first 19 randomly chosen oysters were measured for SH. Spat abundance, which was only measured on a subset of

the seed and/or market oysters in each dredge, was converted to an index that we calculated as the total number of spat counted in each dredge divided by the proportion of oysters examined for spat in that dredge (a maximum of 10 oysters).

Louisiana has 7 widely recognized coastal study areas (CSAs); the analyses were conducted for CSA 1N, CSA 1S, and CSA 3 individually, whereas CSAs 4–7 were combined because these CSAs had relatively few sites (Fig. 1). Most of the sites in CSA 4 are towards its western edge (Fig. 1), where little to no oil was observed using the Shoreline Cleanup Assessment Technique observations (Michel et al. 2013). In Mississippi, only 5 reefs in Mississippi Sound were surveyed consistently over the time period we investigated. However, there were many replicates (14 to 20) per reef that resulted in robust mean abundance measurements.

In Florida, oyster abundances in each size category were calculated from the density estimates supplied by the state and multiplying the overall density by the proportion of oysters in each size category (the proportion of spat oysters was determined by subtracting the percentage of oysters  $\geq 50$  mm from 100%). Finally, the data from Texas and Florida were collected year-round; thus, we limited data from these states to samples collected from August to November to examine only the late-summer reproductive period.

### Statistical analyses

A series of paired *t*-tests were used in each region to test whether the densities of spat-, seed-, and market-sized oysters collected from reefs in the baseline period (2006–2009) differed from the densities of each respective size class in 2010, 2011, and 2012. Due to the differences in how each state sampled oysters and potential regional differences in site characteristics, separate analyses were conducted for the following 7 regions: Galveston Bay, Texas; western Louisiana (CSAs 7–4), CSA 3, CSA 1S, CSA 1N; Mississippi Sound, Mississippi; and Apalachicola Bay, Florida. Within each region, sites were treated as independent replicates and had to have been sampled in the comparison year (2010, 2011, or 2012) and at least 2 out of the 4 baseline years (2006–2009) to be included in these analyses (see Table S1 in the Supplement at [www.int-res.com/articles/suppl/m576p163\\_supp.pdf](http://www.int-res.com/articles/suppl/m576p163_supp.pdf) for a breakdown of which sites were sampled in each year). We created this site selection criterion because 86.3% of sites in Texas were sampled in either 1 or 2 years. Including

Table 1. Size classes of eastern oysters *Crassostrea virginica* (based on shell height in mm) used to analyze the historical density data for each Gulf State

State	Market	Seed	Spat
Texas	75	26–74	5–25 (index)
Louisiana	75	25–74	0–24
Mississippi	75	25–74	0–24
Alabama	75	26–74	0–25
Florida	75	50–74	0–49

only sites that were sampled in at least 3 or 4 years during the baseline period would have significantly reduced our ability to detect differences. Meanwhile, in Louisiana, Mississippi, and Florida, 77% of sites were sampled in all 4 years, and 84% were sampled in 3 or more years of the baseline period.

Because the northern GoM was likely still recovering from hurricanes Katrina and Rita in 2006, we assessed whether oyster densities from 2006 should be included in the baseline period. In particular, we conducted an additional series of analyses in which we excluded the 2006 data and compared the 2 sets of results. Excluding 2006 from the baseline period resulted in almost identical results and had no effect on the overall conclusions of our study. Thus, we decided to include 2006 in our baseline period. For a particular site, the mean density during the baseline period was compared to 2010, 2011, and/or 2012. A p-value of <0.1 was considered significant in accordance with guidance for environmental impact studies (Underwood 1989). All analyses were conducted in R (v. 3.2.3).

We did not adjust the alpha level to avoid spurious results because of the mathematical, logical, and

practical concerns relative to ecological studies raised by Moran (2003). For instance, ecological field studies, and especially environmental impact studies, often have small numbers of replicates due to the financial and logistical challenges associated with achieving additional replication and hence often have low statistical power. Moran (2003) also pointed out an important paradox associated with conducting additional tests when the alpha level is adjusted—the more tests that one conducts, the probability of finding a significant result decreases dramatically.

## RESULTS

In Galveston Bay, Texas, indices of oyster densities were largely similar before vs. immediately after the spill occurred in 2010. This survey region was the farthest away from the spill area (Fig. 1). In particular, indices of oyster densities of all 3 size categories during the baseline period (i.e. 2006–2009) did not differ from those in 2010 in this region (Table 2, Fig. 2). In-

Table 2. Paired *t*-test results for spat-, seed-, and market-sized eastern oysters *Crassostrea virginica* by state or region. For each analysis, n (the number of paired sites compared in the analysis), p-value (significant values at  $p < 0.10$  are in **bold**), and the percent difference of the baseline (2006–2009) less the comparison year (2010, 2011, or 2012) divided by the baseline (% Diff) are displayed. Alabama (Mobile Bay) data are not included because sample sizes were too small to provide meaningful comparisons. TX: Texas, LA: Louisiana, CSA: coastal study area, FL: Florida

Region	Spat			Seed			Market		
	n	p	% Diff	n	p	% Diff	n	p	% Diff
2010									
Galveston Bay, TX	25	0.11	127.8	25	0.33	16.3	25	0.44	-4.6
LA CSA 7-4	22	0.23	-35.8	22	0.19	-27.0	22	<b>0.09</b>	-52.4
LA CSA 3	6	<b>0.02</b>	-86.9	6	<b>0.09</b>	-78.9	6	0.27	42.7
LA CSA 1S	29	<b>&lt;0.001</b>	-96.1	29	<b>&lt;0.001</b>	-80.3	29	<b>&lt;0.001</b>	-79.0
LA CSA 1N	12	<b>0.02</b>	-85.7	12	<b>0.04</b>	-87.1	12	<b>0.01</b>	-76.6
Mississippi	5	<b>0.03</b>	-77.4	5	<b>0.002</b>	-80.8	5	<b>0.01</b>	-80.5
Apalachicola Bay, FL	6	0.16	-20.6	6	0.11	-37.5	6	<b>0.04</b>	-56.1
2011									
Galveston Bay, TX	23	<b>0.02</b>	-73.3	23	0.32	-18.4	23	0.47	5.6
LA CSA 7-4	28	<b>0.01</b>	-49.8	28	<b>0.02</b>	-52.7	28	<b>0.05</b>	-52.1
LA CSA 3	6	0.12	71.5	6	0.11	149.0	6	0.30	45.1
LA CSA 1S	29	<b>&lt;0.001</b>	-99.0	29	<b>&lt;0.001</b>	-97.5	29	<b>0.001</b>	-66.1
LA CSA 1N	13	<b>0.01</b>	-95.1	13	<b>0.03</b>	-89.5	13	<b>0.004</b>	-92.4
Mississippi	5	<b>0.01</b>	-84.3	5	<b>0.02</b>	-45.8	5	0.12	100.6
Apalachicola Bay, FL	5	0.26	-21.0	5	0.15	-31.6	5	<b>0.02</b>	-44.1
2012									
Galveston Bay, TX	26	0.12	-51.6	26	0.34	27.8	26	0.33	26.1
LA CSA 7-4	28	<b>0.003</b>	-56.0	28	<b>0.001</b>	-69.0	28	<b>0.01</b>	-78.7
LA CSA 3	6	0.15	112.9	6	0.37	-12.2	6	0.20	235.4
LA CSA 1S	29	<b>&lt;0.001</b>	-86.9	29	<b>&lt;0.001</b>	-99.5	29	<b>&lt;0.001</b>	-84.7
LA CSA 1N	13	0.10	-47.3	13	<b>0.05</b>	-86.0	13	<b>0.01</b>	-90.0
Mississippi	4	0.17	25.2	4	<b>0.01</b>	-71.3	4	0.19	-16.6
Apalachicola Bay, FL	5	<b>0.07</b>	-60.9	5	<b>0.01</b>	-86.6	5	<b>0.01</b>	-70.0

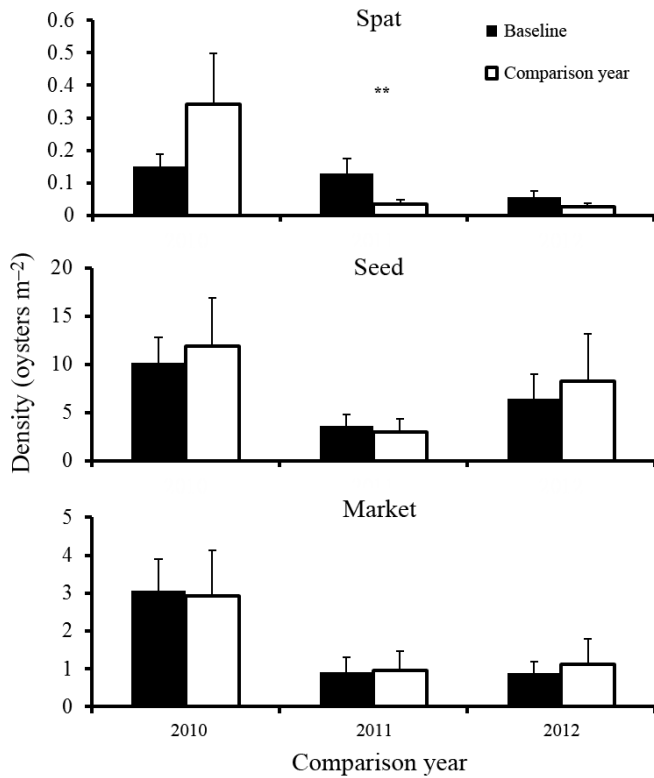


Fig. 2. Pairwise comparisons of spat-, seed-, and market-sized eastern oyster *Crassostrea virginica* densities (oysters  $m^{-2}$ ) at sites in Galveston Bay, Texas, during the baseline period (2006–2009) vs. 2010, 2011, and 2012. Significant *t*-tests are identified at \* $p < 0.1$ , \*\* $p < 0.05$ . Error bars indicate +1 SE

dices of spat, seed, and market oyster densities also did not change in either 2011 or 2012 relative to the baseline period other than lower spat in 2011.

Densities of spat and seed oysters directly after the spill in 2010 did not differ from the baseline period in western Louisiana (CSAs 7–4), which was at the far western edge of the spill footprint (Table 2, Fig. 3). Densities of market-size oysters in this region were significantly lower in 2010 than the baseline period. In this region, oyster densities in 2011 and 2012 were significantly lower than those in the baseline period for all 3 size classes.

Most of the remaining regions that were within the footprint of the spill had lower spat, seed, and adult oyster densities immediately after the spill in 2010 than the baseline period, and differences between the 2 periods were greatest east of the Mississippi River in Louisiana (Fig. 1). Spat and seed densities were significantly lower at the western edge of the spill in Louisiana's CSA 3 in 2010 relative to the baseline period, but did not differ in 2011 and 2012 (Table 2, Fig. 4). The density of market oysters in this

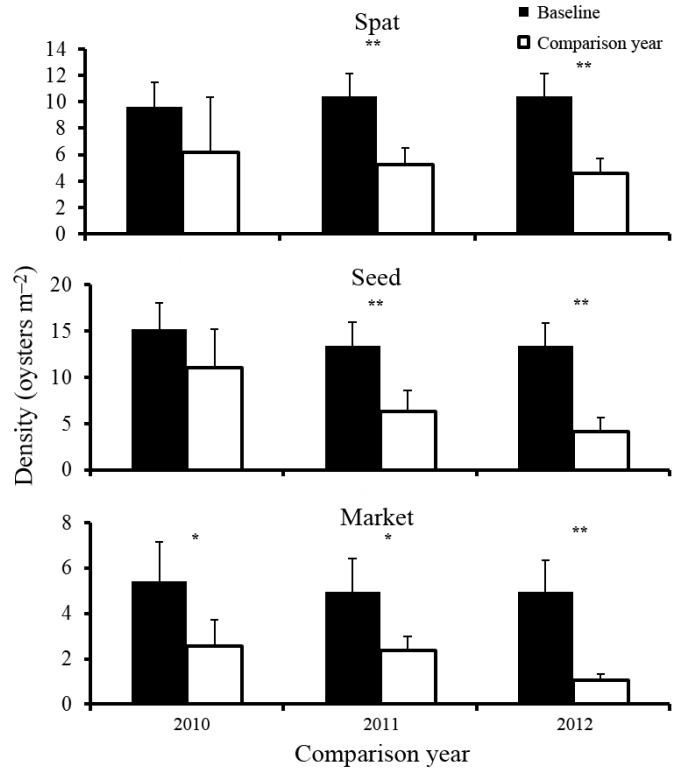


Fig. 3. As in Fig. 2, but for CSAs 7–4, Louisiana

region did not change substantially until it increased by over 200% in 2012.

Densities of all size classes of oysters sampled east of the Mississippi River in Louisiana were significantly lower and persistently low throughout the post-oiling monitoring period. In CSA 1S, densities of all 3 size classes were 79–96% lower in 2010 relative to the baseline period (Table 2, Fig. 5). Furthermore, all 3 size classes within CSA 1S remained significantly (85–100%) lower than the baseline period through 2012, with spat and seed oysters largely absent from almost all quadrat samples. In CSA 1N, densities of all 3 size classes of oysters were 77–87% lower in 2010 relative to the baseline period, and seed and market densities remained significantly lower through 2012 (Table 2, Fig. 6). Spat levels in CSA 1N were extremely low in 2011 but did not significantly differ from the baseline period in 2012 even though there was a trend of fewer spat during this period ( $p = 0.10$ ).

In Mississippi, densities of all 3 size classes of oysters were lower in 2010 by 77–81% relative to the baseline period (Table 2, Fig. 7). In 2011, densities of spat in Mississippi Sound remained significantly lower than the baseline period, whereas the 2011 density of market oysters did not differ from the base-



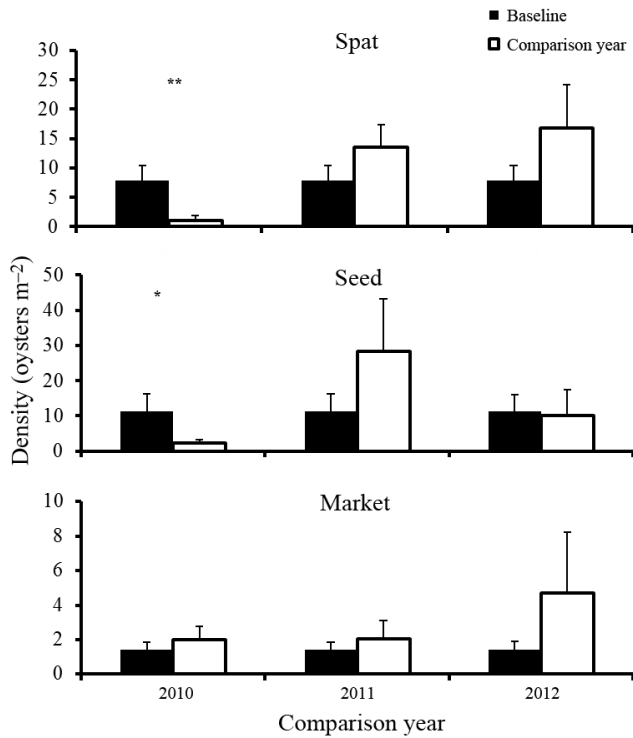


Fig. 4. As in Fig. 2, but for CSA 3, Louisiana

line period. In 2012, only densities of seed oysters in Mississippi were significantly lower than the baseline period.

At the far eastern extreme of the oiling footprint in Apalachicola Bay, market oyster densities in 2010 were significantly lower by 56% than those for the baseline period, whereas spat and seed oyster densities did not differ between the baseline and 2010 (Table 2, Fig. 8). Market oyster densities in 2011 and 2012 were also significantly lower than the baseline period. Meanwhile, spat and seed oyster densities in 2011 did not differ from the densities of those size classes in 2011. However, both of these size classes were significantly lower in 2012 than they were in the baseline period.

## DISCUSSION

Although the fisheries-independent oyster survey methods used by state resource agencies from Texas to Florida differ, collectively they offer critical baseline and post-impact information on oyster populations from within and adjacent to the DWH spill impact area. Specifically, they provide an opportunity to evaluate the potential impacts of an unprecedented spill on a critical estuarine habitat that is val-

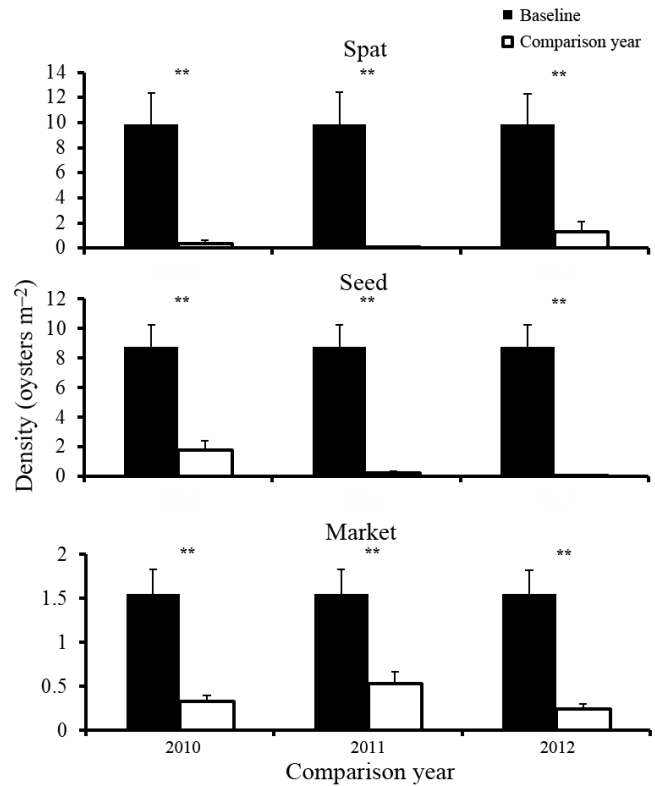


Fig. 5. As in Fig. 2, but for CSA 1S, Louisiana

ued not only as a harvestable oyster resource that can be extracted, but also for the ecosystem services that intact oyster reefs provide, such as stabilizing shorelines, removing anthropogenic nitrogen, and providing habitat for juvenile and adult finfish and crustaceans (Coen et al. 1999, Peterson & Lipcius 2003, Peterson et al. 2003, Piazza et al. 2005, Piehler & Smyth 2011, Grabowski et al. 2012). The results from these surveys collectively suggest that declines in oyster densities were typically greatest and most persistent in the coastal regions with highest oiling from the DWH spill and associated response activities such as the release of freshwater.

The opening of the Caernarvon and Davis Pond freshwater diversions in 2010 in response to the spill resulted in prolonged periods of low salinity (<3‰) in much of CSAs 1S and 3, respectively, in Louisiana (Fig. 9; Rouhani & Oehrig 2015). Prolonged exposure to low salinity (<5‰) can induce mortality in oysters and can inhibit feeding, growth, and spawning (Loosanoff 1953, Kennedy et al. 1996, Powers et al. 2017a, this Theme Section). Powers et al. (2017a) modeled the effect of consecutive days of exposure to <5‰ between April and November on oyster survival using experimentally deployed oysters throughout southern

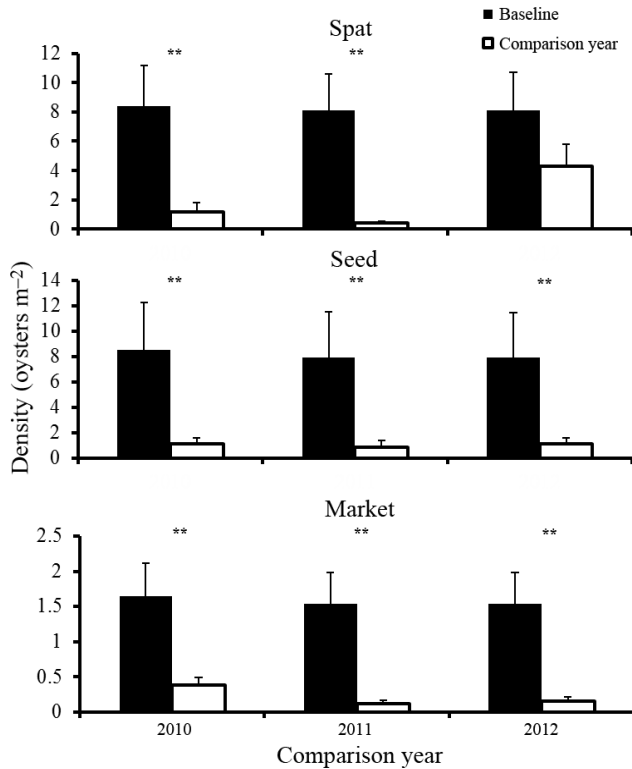


Fig. 6. As in Fig. 2, but for CSA 1N, Louisiana

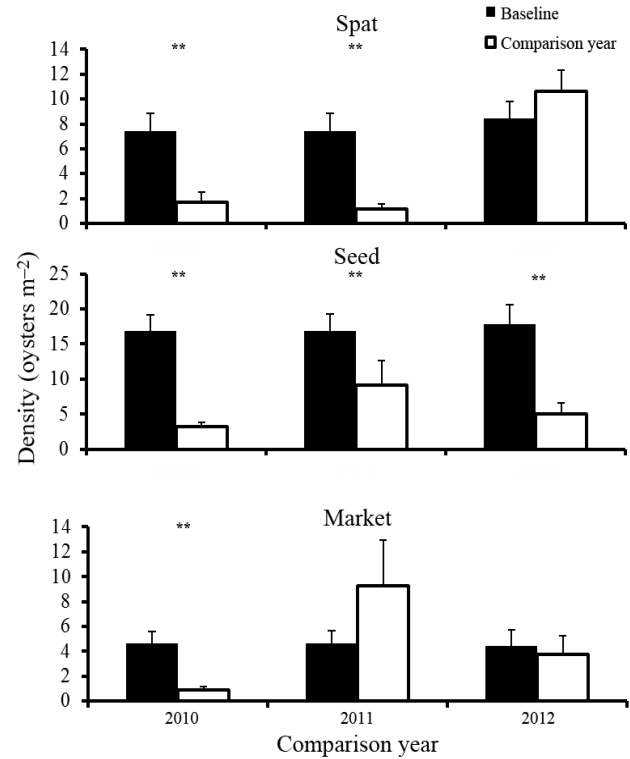


Fig. 7. As in Fig. 2, but for Mississippi Sound, Mississippi

Louisiana that were exposed to differing levels of low salinity. They found a sharp initial decline in oyster survival, such that <40% of oysters survived greater than 25 consecutive days of exposure to low salinity. Meanwhile, Loosanoff (1953) revealed that oysters stopped feeding and gametogenesis at 3‰, and that oysters of different ages including spat were equally vulnerable to low salinity. While the optimum salinity and salinity range of egg and larval development is likely influenced by the conditions experienced by the parents during gametogenesis, these early life-history stages may be more vulnerable than older oysters to low salinity (Davis 1958, Kennedy et al. 1996). For instance, Davis (1958) found that adults that were held at 9‰ produced zygotes that developed at salinities >7.5‰, and larvae from parent oysters living in higher (26–27‰) salinity water exhibited limited growth at 7.5‰. The Caernarvon freshwater diversion coincides with the region of the GoM east of the Mississippi River (i.e. eastern Louisiana through Mississippi) where declines in oyster densities were greatest, with >80% declines in almost all categories of oysters persisting 2 yr after the spill in eastern Louisiana.

Immediately after the spill in 2010, there were declines in spat and seed oysters in CSA 3, but not

adult oysters, and these declines in juvenile oysters did not persist beyond 2010. This failure to detect impacts to adult oysters in 2010 and all size classes beyond 2010 could stem in part from the low number of sites (6) that were surveyed in CSA 3, with all but 1 site being highly aggregated in the western portion of the region (Fig. 1). In contrast, 29 sites in CSA 1S met the criteria for inclusion in our analyses, and they were dispersed throughout this region. Impacts along the edge (i.e. western Louisiana and Apalachicola Bay, Florida) of the spill footprint (i.e. where oil-on-water and shoreline oiling occurred) were less severe. For instance, significant declines in market-size but not juvenile oysters were observed in 2010 in CSAs 7–4 in Louisiana and Apalachicola Bay, Florida. Furthermore, indices of oyster abundances in Texas, which is west of the spill footprint, did not decline in 2010 immediately after the spill occurred.

The decline in oyster abundances that the state surveys revealed throughout the oil spill area may be in part a response to other environmental impacts that occurred during the post-spill period: First, the opening of the Bonnet Carre and Morganza spillways in 2011 to alleviate pressure on the levee system in Louisiana has been posited as a potential

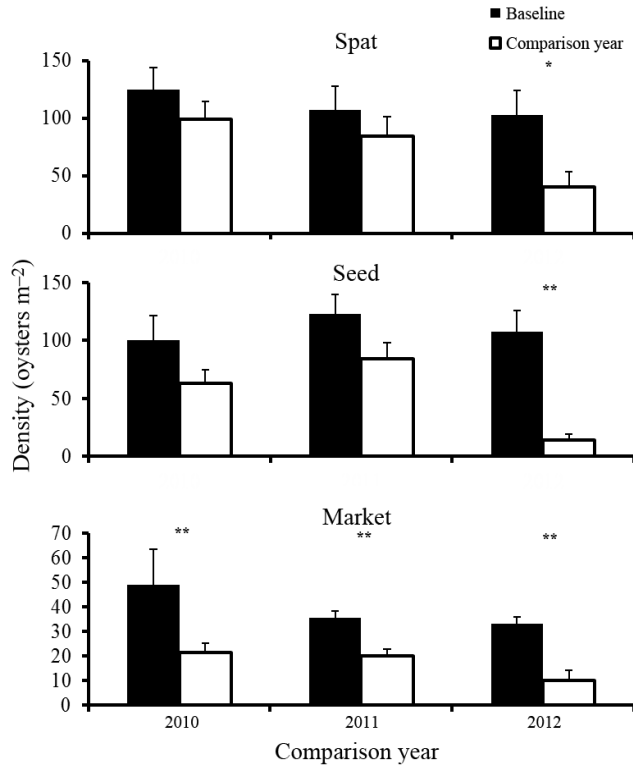


Fig. 8. As in Fig. 2, but for Apalachicola Bay, Florida

source of oyster mortality (Rouhani & Oehrig 2015). Given that continued exposure to salinity <5‰ can induce mortality (Powers et al. 2017a), these freshwater releases may have induced additional mortality. However, the declines in oyster abundances in

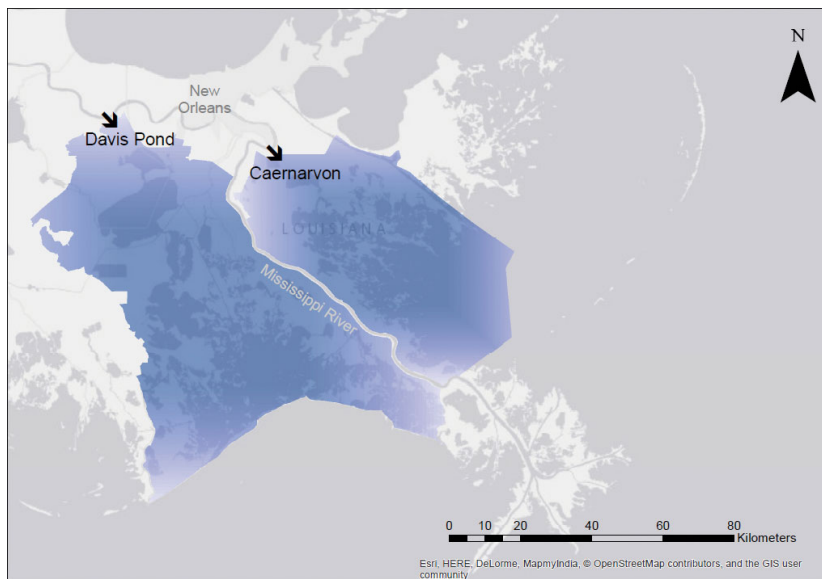


Fig. 9. Caernarvon and Davis Pond freshwater diversions in Louisiana, open in 2010, displaying the direction of freshwater discharge (arrows) and the outfall regions (shaded regions)

the regions impacted by these openings, CSA 1N in Louisiana and Mississippi Sound, largely began occurring in 2010 prior to the opening of these spillways.

Second, when estimating anthropogenic impacts from oiling and other activities on harvested species, it is important to consider if changes in fishing pressure may have influenced impacted populations. In 2010, Louisiana, Mississippi, and Alabama closed their coastal waters to oyster harvesting in response to the threat of oiling, which consequently reduced oyster fishing mortality in these regions. In contrast to these oil-induced closures, the commercial fisheries in Florida and Texas remained open in 2010. Adult oyster abundances may have declined in Florida in 2010 after the spill in part as a result of increased fishing in this region to compensate for reduced harvesting in the closed regions and in anticipation of a looming anthropogenic perturbation even though no evidence of oiling has been found in Apalachicola Bay (Havens et al. 2013, Camp et al. 2015). Collectively, our results suggest that the magnitude of impact from the oiling and response activities might be underestimated since fishing decreased in the most heavily impacted areas relative to previous years and after the spill in less impacted areas (Galveston Bay, Texas, and Apalachicola Bay, Florida).

Third, oyster population dynamics in the GoM and elsewhere are largely influenced by salinity. Because higher salinities within estuaries increase oyster diseases, parasites, and predators, intermediate salinities are thought to promote higher oyster survival and potentially greater fishery productivity (Wilber 1992, Buzan et al. 2009, Seavey et al. 2011, Garland & Kimbro 2015; but see Turner 2006, 2009). The Apalachicola Bay oyster fishery collapsed in 2012 likely as a consequence of consistent low river flow leading to increased salinity, increased oyster parasites and predators, elevated oyster mortality, and eventual recruitment failure and population collapse (Oczkowski et al. 2011, Petes et al. 2012, Havens et al. 2013, Camp et al. 2015). Even after accounting for this and other confounding factors that differed among regions, we found elevated levels of oyster mortality after the DWH oil spill predominately in the regions that were most heavily impacted by it.

Human activities have been disturbing ecosystems globally for millennia, which challenges efforts to establish pre-disturbance baselines (Jackson et al. 2001). The GoM experienced several natural and anthropogenic disturbances in the decade prior to DWH, including droughts and hurricanes Katrina and Rita. Many of the state surveys of oyster densities extend well before the 4 yr period that we selected, but we chose this period as a baseline that included both wet and dry years. It is important to include longer time periods to estimate baseline levels due to the interannual variability in abiotic and biotic processes that influence the population dynamics of marine species. Conversely, selecting data from an individual year may not be indicative of future oyster performance, thereby potentially biasing estimates of change in oyster densities after the DWH event. We excluded data collected before 2006 in our baseline period due to hurricanes Katrina and Rita. These storms altered the environment to such a magnitude that data collected before 2006 may not be an appropriate baseline for conditions prior to the DWH event. Although the fisheries-independent surveys that were used in this study focused on subtidal oyster reef habitat, similar impacts may have transpired on intertidal oyster reefs in these regions where oil impacts and response activities were highest. However, differences in the physical environments of intertidal and subtidal oyster reefs in part explain why submergence largely influences oyster growth, recruitment, disease susceptibility, and survival (Fodrie et al. 2014, Solomon et al. 2014). Thus, intertidal reefs may respond to and recover from anthropogenic stressors such as oil spills very differently than do subtidal reefs. This difference may explain why Dietl & Durham (2016) found that the average size of living and dead oysters did not differ between oiled and unoled intertidal reefs. Yet, Powers et al. (2017b, this Theme Section) found that impacts of oiling resulted in the reduction of oyster habitat by up to 77% in areas that experienced heavy oiling. Thus, while oiling may not have affected the average size of living oysters, degradation of this important habitat likely resulted in the loss of important ecosystem services.

In conclusion, we found that significant declines in all size-classes of oysters occurred in eastern Louisiana through Mississippi. These regions experienced declines in oyster densities that largely did not recover during the post-spill period monitored in this study. Moreover, continued low recruitment coupled with low densities of adult oysters well after the spill could indicate that recruitment failure is occurring

in this region. Because each GoM state conducts fisheries-independent surveys, spatial and temporal data were available to compare before and after the spill in control and impacted areas, similar to a BACI design. These surveys are extremely valuable for protecting state public resources such as oyster reef habitat from unexpected impacts like the DWH oil spill.

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Contribution to the Theme Section

'Response of nearshore ecosystems to the Deepwater Horizon oil spill'



# Consequences of large-scale salinity alteration during the *Deepwater Horizon* oil spill on subtidal oyster populations

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**ABSTRACT:** Response actions associated with oil spills often have significant impacts on ecological communities. During the 87 d long *Deepwater Horizon* oil spill, the State of Louisiana (USA) released vast quantities of Mississippi River water into 2 estuarine basins (Barataria Bay and Black Bay/Breton Sound) in response to the approach of oil. We assessed the impact on subtidal oyster populations of this novel oil spill response action using 3 independent methods: (1) comparison of fisheries-independent post-spill densities to a pre-spill temporal baseline; (2) comparison of oyster density collected during natural resource damage assessment sampling between the area of maximal freshwater impact and reference areas in the 2 basins; and (3) estimation from a dose-response model derived from an analysis of an *in situ* mark and recapture study conducted in 2010 to assess the relationship between salinity and oyster mortality. A substantial portion of both basins (483 km<sup>2</sup> of Barataria Bay and 362 km<sup>2</sup> of Black Bay/Breton Sound) experienced prolonged periods of very low (<5 ppt) salinity in 2010 that lasted at least 1 mo longer than the average duration of low salinity between 2006 and 2009. The 3 approaches all indicate that dramatic losses occurred in the number of market-sized (>75 mm) oysters as a result of a system-wide lowering of salinities, with an estimated 1.16 to 3.29 billion market-equivalent oysters lost. The efficacy of the large-scale response action of altering hydrographic conditions during the summer oyster growth period should be examined in light of the major perturbation to oyster communities.

**KEY WORDS:** Estuary · Oil spill response · Natural resources damage assessment · Oyster reefs · Gulf of Mexico · *Crassostrea virginica* · Hydrography

## INTRODUCTION

Eastern oysters *Crassostrea virginica* are a critical component of many estuarine ecosystems, particularly those along the US Gulf and mid-Atlantic coasts (Beck et al. 2011), and are the quintessential estuarine-dependent species, spending their entire life cycle within estuaries. Although eastern oysters have been

observed across a large range of salinities (3–36 ppt), they generally thrive in the narrow band of mesohaline water in an estuary (Melancon et al. 1998). This 'green band' of water formed by the interface between the sediment-laden, brown freshwater entering from riverine systems and runoff and the blue marine water ingress from the oceanic realm is a constantly fluctuating hydrographic zone of estuaries.

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Too much freshwater reduces local salinity to levels where survivorship, growth, or reproduction of oysters is impaired, whereas too much ocean water generally elevates salinities and is associated with higher levels of predators (e.g. the oyster drill *Stramonita haemastoma*) or disease (e.g. the protozoan parasite *Perkinsus marinus*) that leads to reduced survivorship (e.g. Gunter 1955, Davis 1958, 1979, Chatry et al. 1983, Brown & Richardson 1988, Soniat & Brody 1988, Fodrie et al. 2008, La Peyre et al. 2009, 2013, Soniat et al. 2013). Although the specific thresholds of these triggers varied from study to study, the general pattern of oysters thriving in waters whose average annual salinities fall between 8 and 22 ppt with frequent brief pulses of freshwater input is well established and accepted by oyster biologists. Because settlement of oysters is gregarious on existing oyster shells, sustainable populations of oysters require that the position of this green zone of water stays relatively fixed over time. A substantial perturbation of this salinity zone away from areas of existing oyster shell cover, particularly at a time of increased water temperatures, can both hamper reproduction and result in oyster mortality that can have lasting impacts on the oyster fishery as well as affecting the ecological benefits that accrue from healthy oyster reefs.

On 20 April 2010, the *Deepwater Horizon* (DWH) exploded and set off an unprecedented chain of environmental perturbations in the northern Gulf of Mexico (GoM). Hundreds of millions of liters of oil were spilled into the GoM, resulting in the oiling of at least 2000 km of estuarine and coastal shorelines. In addition to the contaminant release, response actions taken by state and federal resource agencies to mitigate the environmental disaster also introduced potential environmental perturbations. The State of Louisiana, in response to the potential damage from the compromised well head, took the unprecedented action of releasing large quantities of freshwater through diversion structures along the Louisiana coastline to prevent the ingress of oil and to flush contaminants from where oil was likely to come ashore (Martínez et al. 2012). The Davis Pond and Caernarvon diversions were the 2 major diversion structures opened; these structures allow freshwater from the Mississippi River to flow into Barataria Bay and Black Bay/Breton Sound, respec-

tively. The Davis Pond diversion is located on the southwestern bank of the Mississippi River in St. Charles Parish, approximately 24 km (15 miles) upstream of New Orleans (US ACE, New Orleans District; [www.mvn.usace.army.mil/About/Projects/DavisPondFreshwaterDiversion.aspx](http://www.mvn.usace.army.mil/About/Projects/DavisPondFreshwaterDiversion.aspx)), while the Caernarvon diversion is situated on the east bank of the Mississippi River in Plaquemines Parish, downstream of New Orleans (LDNR 2003). The Caernarvon diversion was opened in response to the DWH spill on 23 April 2010 and remained open through the first 2 wk of August at or near maximum capacity ( $\sim 225 \text{ m}^3 \text{ s}^{-1}$ ; Fig. 1). Davis Pond remained open from 8 May through 10 September 2010, with flow ranging from 200 to  $275 \text{ m}^3 \text{ s}^{-1}$ .

Large-scale manipulation of hydrographic features is not a typical response to an oil spill; however, the scale of the DWH oil spill challenged conventional response activities both in the open ocean as well as in estuarine waters. Freshwater diversions are used to divert water from the Mississippi River to help enhance wetland vegetation and re-nourish marsh ecosystems (DeLaune et al. 2003). Normally, large quantities of freshwater are allowed to flow through the structures during the cooler winter and early spring months of each year because of potential impacts to oysters and other fisheries that would result from exposure to lower-salinity waters during the warmer summer months (Turner 2006, La Peyre et al. 2013, Rose et al. 2014). In 2010, these diversions remained opened for significantly longer and at a higher flow rate than in previous years.

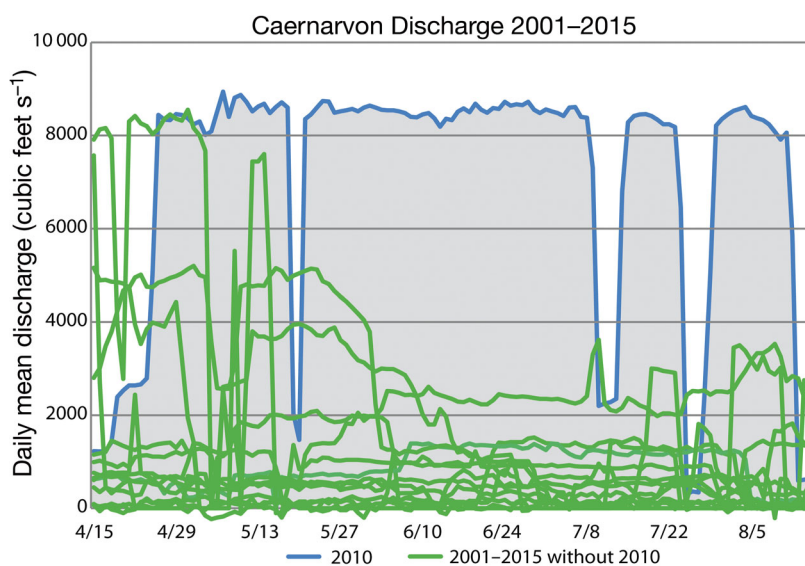


Fig. 1. Discharge of Mississippi River water from the Caernarvon freshwater discharge structure from 2001 to 2015. Dates are given as month/day; 1 cubic foot =  $0.028 \text{ m}^3$



The introduction of substantial quantities of freshwater into an estuarine system, (e.g. due to a major storm event) would be expected to have substantive, albeit short-term (1–2 yr), consequences on oyster fishery resources in the 2 basins. In addition to their role in supporting a valuable US fishery, oysters are essential components of healthy estuarine ecosystems. Hence, changes in the abundance of oysters or the areal extent of oyster reef can have substantial ecological consequences. The suspension-feeding activity of oysters enhances benthic pelagic coupling and removes particulates from the water column, potentially increasing water clarity (see Coen et al. 2007 and Grabowski et al. 2012 for a complete list of ecological services). Reefs formed by the gregarious settlement of oysters provide ecological services including provision of habitat for fish and mobile invertebrates (Peterson et al. 2003), enhanced biogeochemical cycling (Piehler & Smyth 2011), and shoreline stabilization (Scyphers et al. 2011).

As part of the DWH oil spill natural resources damage assessment (DWH NRDA), we performed an extensive assessment of the potential impacts of the large-scale introduction of freshwater on oyster resources in coastal Louisiana. Because the diversions were open as part of the DWH oil spill response activities, damages resulting from the diversion openings would be a legitimate component of the damage assessment, and US federal regulations require the public to be compensated for the damages. We conducted a multi-pronged assessment of the impact of these freshwater diversions on subtidal oyster populations in the northern GoM. Multiple analyses using both state fisheries-independent data and data collected as part of the DWH NRDA showed substantial impacts of the freshwater diversion releases on oyster populations in Barataria Bay and Black Bay/Breton Sound.

## MATERIALS AND METHODS

We estimated injury by comparing oyster density (abundance  $m^{-2}$ ) in areas exposed to the 2010 Davis Pond and Caernarvon diversion openings against baseline oyster densities, where baseline is defined either as historical, pre-spill densities or as densities in post-spill areas unaffected by the diversions or other aspects of the DWH incident. We estimated injury in 2 basins: Barataria Bay basin to the west of the Mississippi River, which receives the outfall from the Davis Pond diversion, and Black Bay/Breton Sound basin to the east, which receives the outfall

from the Caernarvon diversion. We assessed differences in post-spill and baseline densities using multiple methods and datasets to test the robustness and bracket the uncertainty of our injury results. Specifically, we applied 3 approaches to quantify subtidal oyster injury. The first approach compared post-spill densities measured by the Louisiana Department of Wildlife and Fisheries (LDWF) to a pre-spill temporal baseline ('fisheries temporal'). The second approach ('NRDA spatial') employed a spatial definition of baseline conditions, based on modeled estimates of exposure to low salinities in each basin. It assessed oyster density differences between the area of maximal freshwater impact and baseline areas in these basins, using data collected as part of the DWH NRDA in 2010. The third approach ('Nestier tray/NRDA') defined injured and baseline locations using historical modeled salinity conditions and a dose-response model of freshwater impacts on mortality derived from analysis of annual Louisiana Nestier tray studies in the affected basins (Table 1).

### Data sources

#### Louisiana fisheries-independent density data

We obtained oyster densities from diver-collected quadrat samples collected in June and/or July of each year from 2006 to 2010 from the LDWF annual oyster stock assessment program. The sampling program consisted of 8 sites in Barataria Bay and 32 sites in Black Bay/Breton Sound within known reef areas of the public oyster seed grounds (Fig. 2). From 2006 to 2009, site density was characterized using two 1.0  $m^2$  quadrats, except at cultch plant sites, where teams collected five 0.25  $m^2$  quadrats (LDWF 2006, 2007, 2008, 2009, 2010). All density results from 0.25  $m^2$  quadrats were scaled to 1.0  $m^2$ . Beginning in 2010, density was characterized using five 1.0  $m^2$  quadrats at all sites. Samples were collected in July of each year at approximately the same location, with LDWF teams randomly placing quadrats after poling at site coordinates to ensure sampling occurred on oyster habitat. We compiled LDWF density data into 3 size categories: spat (<25 mm), seed (25–75 mm), and market (>75 mm).

#### DWH NRDA density data

As part of the DWH NRDA efforts, oyster density data were collected from July to October 2010. Sam-

Table 1. Summary of injury calculation inputs for the 3 approaches used in this study. NRDA: natural resources damage assessment, LDWF: Louisiana Department of Wildlife and Fisheries

Method	Key data	Year(s) data collected	Baseline	Injury area	Injury calculation
Fisheries temporal	LDWF fisheries abundance data (oysters $m^{-2}$ )	2006–2009, 2010	Based on historical data: avg. oyster densities 2006–2009	Area where oysters are likely to occur	Avg. difference between density in 2010 and historical-baseline $\times$ basin area
NRDA spatial	NRDA abundance data (oysters $m^{-2}$ )	2010	Avg. densities of sites outside of freshwater polygon	FW polygons defined by areas with >30 additional consecutive days with salinity <5 ppt in 2010 compared to historical time periods	Survival difference between avg. density in vs. out of freshwater polygon
	Predicted salinity	2006–2009, 2010	2006–2009		
Nestier NRDA	Louisiana state Nestier tray dataset Predicted salinity NRDA densities (oysters $m^{-2}$ )	2010 2007–2009, 2010 2010	Back-calculated NRDA densities using calculated survival rates at NRDA site locations	Areas that indicated a decrease in survival in 2010 compared to historic periods based on the Nestier dose-response curve	Avg. survival difference $\times$ avg. baseline density

pling consisted of up to 8 diver-collected quadrats at 200  $m^2$  study sites in Barataria Bay (8 sites) and Black Bay/Breton Sound (8 sites; Fig. 2). Because this study focused on subtidal oyster injury, sites where oyster habitat typical of nearshore or intertidal oysters was observed were excluded (<5%). The NRDA sample sites included some of the regularly sampled LDWF stock assessment sites plus additional Barataria Bay sites periodically sampled by LDWF. NRDA quadrat sampling locations were randomly generated from oyster habitat areas identified at these sites following side-scan sonar surveys of each site. Field teams used both 0.25 and 1.0  $m^2$  quadrats, with the larger size used where oyster habitat was less contiguous. Quadrat samples were enumerated by size class for spat-, seed-, and market-sized oysters using the same definitions applied to the LDWF data. Density results from 0.25  $m^2$  quadrats were scaled to estimate density per 1.0  $m^2$ .

#### Louisiana Nestier tray data

We obtained data from annual oyster Nestier tray survival studies conducted by LDWF for the years 2007 to 2012. These studies measured the survival of oysters experiencing various salinity regimes. In January of each year, LDWF deployed 70  $\times$  70  $\times$  7.6 cm Nestier trays on the seafloor at a range of distances from the Davis Pond and Caernarvon freshwater diversion outfalls (Fig. 3). Each tray included 20 seed-sized (25–74 mm shell height) oysters collected from the basin where the tray was deployed. Oysters were affixed to the tray in a grid. After deployment, LDWF examined the oysters on each tray during monthly site visits and recorded data on individual oyster sta-

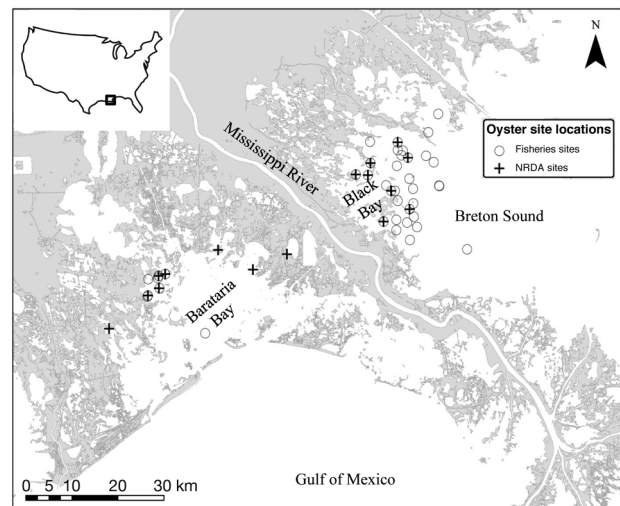


Fig. 2. Oyster sampling locations for the Louisiana Department of Wildlife and Fisheries (LDWF) fisheries data and the natural resources damage assessment (NRDA) studies

tus (alive, dead, or missing; the latter was excluded from our calculations), oyster growth, bottom salinity, and water temperature (P. Banks pers. comm.).

#### Predicted daily salinity

Daily average salinities and temperatures for 2006 through 2012 were estimated on a 200  $\times$  200 m grid-cell basis using a spatio-temporal kriging model (Szpiro et al. 2009, Lindstrom et al. 2011) fit to an extensive database of water quality observations in Barataria Bay and Black Bay/Breton Sound (McDonald et al. 2015). The model employed both continuous (hourly or daily) monitoring data as well as discrete

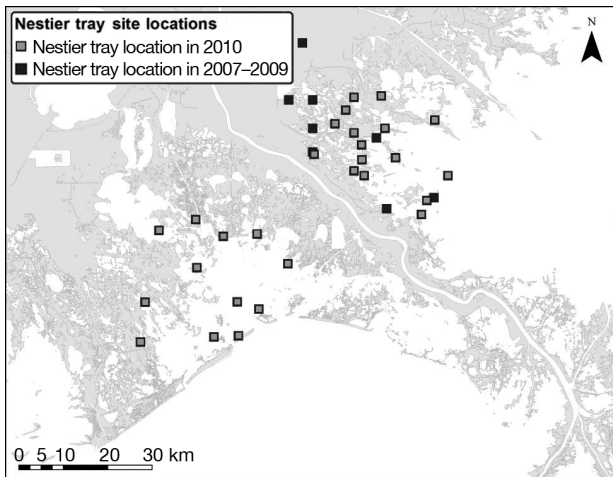


Fig. 3. Geographic extent of sites used in the Nestier tray/natural resources damage assessment (NRDA) approach. The majority of sites display 2010 (light grey) sample locations co-occurring with some 2007–2009 (black) sample locations. See Fig. 2 for further details

(sporadically collected) measurements of water quality from 2006 through 2010, including measurements recorded during DWH NRDA oyster sampling. The model included data collected by LDWF, Louisiana Coastal Protection and Restoration Authority, Louisiana Department of Health and Hospitals, Louisiana Department of Environmental Quality, Mississippi Department of Environmental Quality, and United States Geological Survey (Fig. 4).

#### Percent cover

Percent cover of oyster habitat was estimated for 2 strata within our study area (Fig. 5). One stratum covers 'known/likely oyster habitat,' which includes habitat previously mapped by Louisiana and habitat recommended by state biologists as highly likely to contain productive habitat based on historical harvest activity. The other represents 'possible oyster habitat,' where characteristics such as depth and salinity are sufficient to support oyster habitat, but where no *a priori* evidence of oyster habitat existed. Two sources provided percent cover estimates in the first stratum: 2010 LDWF side-scan sonar mapping in a portion of Black Bay/Breton Sound ('LDWF' stratum), and 2011 DWH NRDA oyster

habitat mapping using poling at randomly selected 200 m<sup>2</sup> sites in both Barataria Bay and Black Bay/Breton Sound (Roman & Stahl 2015). For areas in the possible oyster habitat stratum, percent cover estimates were generated from 2014 side scan sonar surveys conducted by DWH NRDA teams at randomly selected 200 m<sup>2</sup> sites within both basins. For this stratum, percent cover was further stratified based on the presence or absence of oyster leases in the mapping area. Louisiana has an extensive system of leasing sea floor bottom for cultivation of oysters. Lease holders often relay cultch material with oyster spat from high-salinity areas to lease areas for oyster grow out.

#### Injury calculation

The injury calculation approaches we employed require 4 basic steps: (1) establishing baseline conditions of oysters unaffected by the freshwater diversion openings; (2) identifying the areas exposed to freshwater from the 2010 diversion openings; (3) comparing post-spill densities against baseline densities; and (4) scaling spill-related density changes across all oyster habitat in the exposed areas (Table 1).

#### Fisheries temporal assessment

The fisheries temporal approach estimated oyster injury by comparing oyster densities in 2010 in

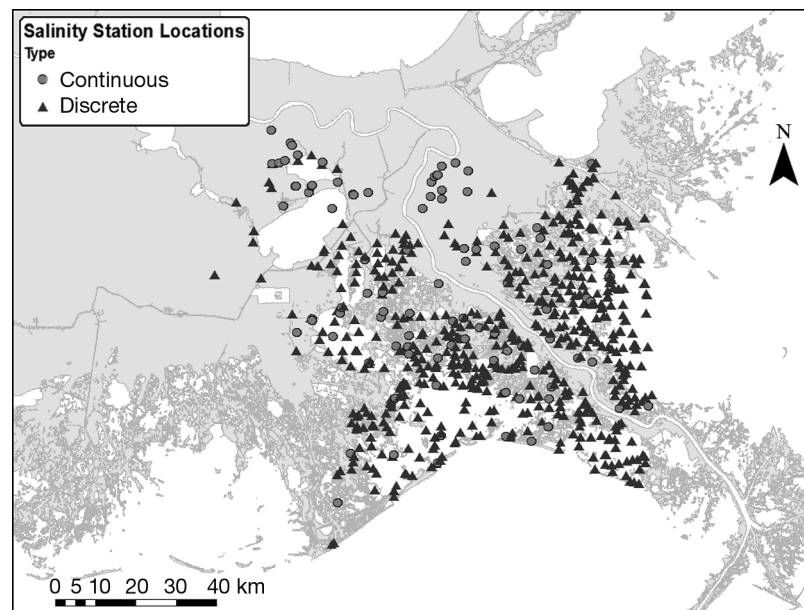


Fig. 4. Salinity sampling and monitoring stations in the study area. See Fig. 2 for further details

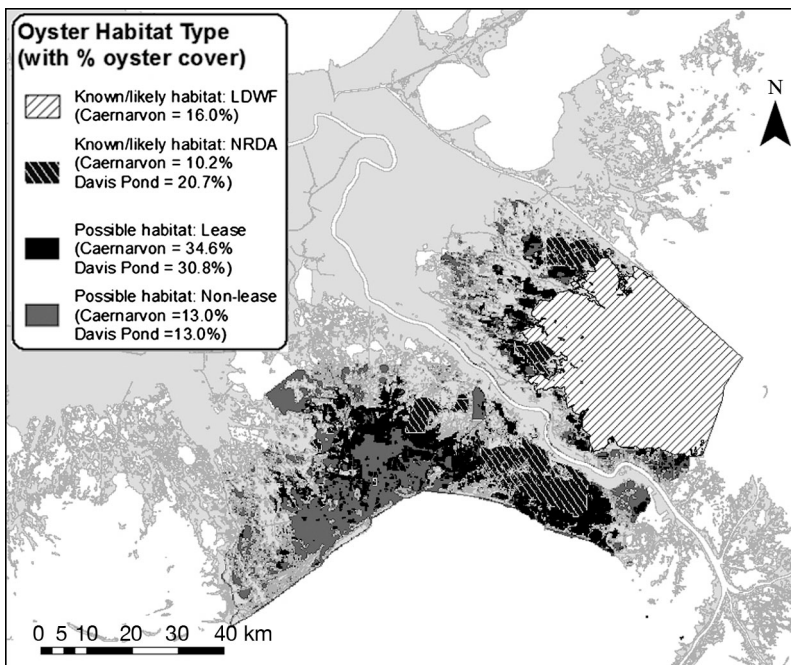


Fig. 5. Geographic extent of oyster strata and estimated reef percent cover. See Fig. 2 for further details

the affected basins to historical (pre-spill) average baseline densities in those basins from 2006 through 2009. Both the 2010 and baseline oyster densities were derived from the LDWF fisheries independent annual monitoring data; the baseline starting year of 2006 was selected so that the baseline would accurately reflect oyster densities following Hurricane Katrina, which could have disrupted or relocated oyster habitat.

The baseline data set for each basin included average densities derived from individual measurements from all LDWF sites in that basin reporting density data in at least 2 of the pre-spill years (6 sites in Barataria Bay and 29 sites in Black Bay/Breton Sound). Differences in densities for spat-, seed-, and market-sized oysters were calculated for each basin separately, scaled to the corresponding injury area, and then summed. Oyster losses were scaled by first estimating the area of oyster habitat in  $m^2$  in each basin and then multiplying that habitat area by the average density loss in oysters  $m^{-2}$ . The area of oyster habitat is estimated by multiplying the area of each sampling stratum shown in Fig. 5 by the corresponding percentage of area in that stratum covered by oyster habitat and then summing across strata. The general formula for this calculation was:

$$\text{Oyster loss} = (\text{Density}_{(2006-2009)} - \text{Density}_{2010}) \times \sum \text{Area}_{\text{stratum}} \times \% \text{cover}_{\text{stratum}} \quad (1)$$

This calculation was performed for each size class. We also calculated an overall number of 'market equivalent' oysters, by assuming 56% of the lost seed oysters and 30% of the lost spat would have survived to market size, based on mortality functions derived from data in Soniat et al. (2012, 2014) and Duke (2008). We then added those losses to the lost market oysters to obtain a total market equivalent oyster loss. As a lower bound on this type of analysis, we also estimated the fisheries temporal-based injury using only the fisheries-independent sites that fell within the low-salinity impact polygon described in the NRDA spatial assessment approach below.

#### NRDA spatial assessment

The NRDA spatial assessment approach delineated the exposed and baseline areas based on the modeled average daily salinity estimates described above. The injury was estimated by comparing densities observed during NRDA sampling events within and outside of the low-salinity exposure areas in each basin in 2010. Both baseline and injured area densities were measured as part of the DWH NRDA in 2010.

We used the results of the spatio-temporal salinity model to delineate polygons in both Barataria Bay and Black Bay/Breton Sound that describe areas of substantial freshwater impact in 2010 relative to historical conditions. Exposures were determined based on interpolated average daily salinities between 27 April and 15 September. This is when the freshwater from the diversions would have reached the study area, and is therefore considered the 'critical time' period. For each 200  $m^2$  grid cell in the salinity model, the maximum number of consecutive days below 5 ppt during the critical time period was calculated for each year between 2006 and 2010. Brief periods when average daily salinities were above 5 ppt for less than 3 d were ignored in the computation of maximum number of consecutive days; such events were not common in the data set. For each grid cell, the maximum number of consecutive days was averaged for the years 2006 to 2009 as the 'historical condition.' If the difference between the historical condition and the 2010 average was more than 30 d, the grid cell was considered affected. Thus, the



polygon of all affected grid cells represents the area with a significant increase in prolonged low-salinity exposure in 2010. The difference of 30 d was selected to maximize the difference between average salinities inside and outside the polygon in 2010, thereby representing the greatest low-salinity impact.

For each size class in 2010, we calculated the difference in oyster densities between areas inside (exposed) and outside (baseline) of the impact polygons described above. The seed-size oyster density in Barataria Bay was higher inside the freshwater-impact polygon than outside. This value was considered as ‘no injury’ and treated as a 0 in the subsequent injury analysis. Oyster loss was scaled by adjusting the injured area for percent cover of oyster habitat. We employed the following general equation:

$$\text{Oyster loss} = (\text{Density}_{\text{out}} - \text{Density}_{\text{in}}) \times \sum \text{Injured area}_{\text{stratum}} \times \% \text{ cover}_{\text{stratum}} \quad (2)$$

Seed and spat losses were scaled to market equivalents as described in the fisheries temporal approach and summed with the lost market oysters.

#### Nestier tray/NRDA assessment

Data from annual oyster Nestier tray studies conducted by LDWF were used to develop dose-response curves linking oyster survival with freshwater exposures during the months of April to September for Nestier tray locations in both Barataria Bay and Breton Sound basins. Doses were measured as the maximum number of consecutive days of salinity below 5 ppt over the April to September time period at each Nestier tray location. This was computed using the year-specific spatial-temporal model results for the grid cell closest to each Nestier tray location.

We limited dose-response modeling to Nestier tray sites with at least 50% oyster survival as of the April status assessment in each given year to avoid including sites with high mortality due to factors other than summer freshwater exposure. In addition, 4 Barataria Bay Nestier sites classified as marine (~10% of sites) were excluded, as these sites may have experienced increased mortality resulting from enhanced predation or increased disease due to their more saline waters (Gunter 1955). We developed dose-response curves for the period from 2007 through 2012, (excluding 2010), as well as for 2010 only. In the curve-fitting process, we set the dose-response curves to 100% survival at the 0 consecutive days of low salin-

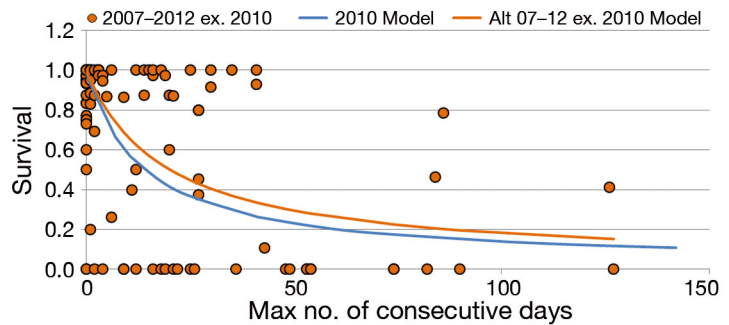


Fig. 6. Dose-response curve demonstrating the relationship between oyster survivorship and number of days below 5 ppt salinity. ex.: excluding

ity. We found little difference between the fitted dose-response curves developed for specific years; thus, our analysis uses the 2010 curves, since those represent relationships between salinity and survival in the year of interest (Fig. 6).

Using the 2010 dose-response curves and results from the salinity model, the decrease in the oyster survival rate in each 200 m<sup>2</sup> grid cell due to the 2010 freshwater diversion exposures was calculated as the difference between survival rate based on the maximum number of consecutive low-salinity days in 2010 and the survival rate based on the average historical (2006–2009) maximum of consecutive low-salinity days in the same cell. Grids with higher predicted survival in 2010 than prior years were assumed to have a 0 decrease in survival. Declines in the survival rate were then averaged across all grid cells within each basin and oyster habitat stratum.

Baseline oyster density values were derived from NRDA quadrat oyster density results collected in 2010. The survival difference ( $\Delta$ Survival) for each NRDA quadrat location was calculated from the dose-response curves as described above. The pre-spill density values for each NRDA quadrat location were estimated using the following equation:

$$\text{Density}_{\text{pre-spill}} = \frac{\text{Density}_{2010}}{1 - \Delta \text{Survival}} \quad (3)$$

Baseline oyster densities were then computed as the average estimated pre-spill density per basin.

The number of oysters lost per m<sup>2</sup> was determined as the product of the average change in the survival rate in 2010, the area of oyster habitat experiencing decreases in survival in 2010, and the average baseline density estimated from the NRDA sites. We used the following general equation to calculate the oyster loss:



$$\text{Loss} = \Delta\text{Survival} \times \text{Density}_{\text{baseline}} \times \sum(\text{Impacted area}_{\text{stratum}} \times \% \text{ cover}_{\text{stratum}}) \quad (4)$$

Losses were estimated by size class and converted to market equivalent oysters in the same manner described above.

## RESULTS

### Salinity area of impact

Major changes in the hydrographic regime of both the Barataria and the Back Bay/Breton Sound basins were observed during the summer of 2010. The change in the number of consecutive days where mean salinity fell below 5 ppt was dramatic: on average, locations in the study area averaged 20.3 additional consecutive days of very low salinity (<5 ppt)

in 2010, compared to average historical values, with a maximum of 138 d. The vast majority of the mesohaline areas of both basins experienced extended periods (>30 d) of salinity <5 ppt. The extent of the area of freshwater impact, defined as areas experiencing more than 30 consecutive days of salinities <5 ppt in 2010 when compared to historical baseline salinities, covered 483 km<sup>2</sup> in Barataria Bay and 362 km<sup>2</sup> in Black Bay/Breton Sound. When exposure is measured in terms of total days <5 ppt (i.e. not necessarily consecutive), the area of influence expands to 528 km<sup>2</sup> in Barataria and 424 km<sup>2</sup> in Black Bay/Breton Sound.

### Oyster losses

All 3 approaches to estimating oyster injury found substantial losses of oyster populations in the sub-

Table 2. Affected area, reef percent cover, and oyster densities by approach and basin. FW: freshwater, FID: fisheries-independent data, OH: oyster habitat, LDWF: Louisiana Department of Wildlife and Fisheries, NRDA: natural resources damage assessment

Quantification approach (5 ppt threshold)	Basin	Stratum	Affected area (km <sup>2</sup> )	% oyster cover	Baseline density (oysters m <sup>-2</sup> )			Post-spill density (oysters m <sup>-2</sup> )			Decrease in density due to FW impact (oysters m <sup>-2</sup> )		
					Spat	Seed	Market	Spat	Seed	Market	Spat	Seed	Market
Nestier tray	Black Bay/Breton Sound	Known/likely OH	44.4	10	0	17.0	8.8	0	11.8	6.1	0	5.2	2.7
		Possible OH (lease)	90.3	35				0	13.2	6.9	0	3.8	2.0
		Possible OH (non-lease)	94.8	13				0	14.3	7.4	0	2.7	1.4
		LDWF	361.4	31				0	13.9	7.2	0	3.1	1.6
		Outside OH	108.8	0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0
	Barataria Bay	Known/likely OH	184.0	21	5.8	41.7	32.0	4.8	34.7	26.7	1.0	7.0	5.4
		Possible OH (lease)	302.9	31				4.4	32.0	24.5	1.4	9.8	7.5
		Possible OH (non-lease)	279.6	13				4.4	31.4	24.1	1.4	10.3	7.9
		Outside OH	659.1	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	FID	Black Bay/Breton Sound	Known/likely OH	75.2	10	9.9	8.8	1.5	0.4	1.7	0.3	9.5	7.0
Possible OH (lease)			107.6	35									
Possible OH (non-lease)			135.4	13									
LDWF			953.1	16									
Barataria Bay		Known/likely OH	189.6	21	7.9	11.4	1.4	1.0	2.4	2.0	6.9	9.0	-0.6
		Possible OH (lease)	363.6	31									
		Possible OH (non-lease)	547.6	13									
NRDA	Black Bay/Breton Sound	Known/likely OH	31.4	10	0	19.8	9.5	0.0	3.7	3.1	0.0	16.1	6.4
		Possible OH (lease)	81.0	35									
		Possible OH (non-lease)	76.7	13									
		LDWF	91.3	25									
		Outside OH	81.2	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Barataria Bay	Known/likely OH	16.4	21	5.7	27.0	24.3	3.2	31.2	20.6	2.5	0 <sup>a</sup>	3.7
		Possible OH (lease)	78.3	31									
		Possible OH (non-lease)	104.5	13									
		Outside OH	284.0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

<sup>a</sup>The seed-size oyster density in Barataria Bay increased, according to the NRDA sample data. This value was considered as 'no injury' and treated as a 0 in the injury analysis

Table 3. Estimates of oysters killed as a result of lowered salinities during summer of 2010 by approach and oyster size class. NRDA: natural resources damage assessment, FW: freshwater

Consecutive days approach	FW	Oyster losses in 2010 ( $10^6$ oysters)				
		Market + seed	Market	Seed	Spat	Market equivalent
Fisheries temporal assessment	—	3642	130	3512	3573	3169
NRDA spatial assessment	5 ppt	1431	560	1025	101	1164
Fisheries in freshwater polygon	5 ppt	1164	142	1022	1389	1131
Nestier tray/NRDA	5 ppt	3543	1465	2078	216	2694

tidal freshwater impact zone in 2010 (Tables 2 & 3). The simplest approach, fisheries temporal, demonstrated a substantial decline in oysters in 2010 compared to pre-spill years. Compared to baseline values derived from the measured average densities in summer quadrat surveys from 2006 to 2009, oyster densities decreased in 2010 by 70% (market) to 100% (seed and spat) in Black Bay/Breton Sound (Table 2). Similar density declines were seen for spat and seed in Barataria Bay; however, market-sized oysters increased slightly ( $+0.6$  oyster  $m^{-2}$ ). These changes in density over the large area of likely oyster habitat (mapped reefs, lease areas, and non-lease areas outside mapped resource) in Barataria Bay ( $1101$   $km^2$ ) and Black Bay ( $1271$   $km^2$ ) resulted in the loss of an estimated  $3.2 \times 10^9$  market equivalent oysters (Table 3), where market equivalent oysters are the sum of market-sized oysters lost plus the lost spat and seed that would be expected to survive to market size over the next 1 or 2 yr. Restricting our extrapolation of injury to the portions of the 2 basins that lie within

the 5 ppt injury polygons resulted in smaller injured areas ( $483$   $km^2$  for Barataria Bay and  $362$   $km^2$  for Black Bay/Breton Sound). The resulting lower bound injury estimate in market equivalent oysters was  $1.1 \times 10^9$  market equivalent oysters (see 'Fisheries in freshwater polygon' in Table 3).

The results for the NRDA spatial approach reflect the oyster abundance differences between NRDA sampling sites located inside and outside the modeled freshwater impact polygons (Fig. 7). Overall, they show an extensive decline in oyster populations across all size classes and in both assessed basins. Within these polygons, densities of seed and market-sized oysters decreased in Black Bay/Breton Sound, and densities of spat and market-sized oysters decreased in Barataria Bay (Table 2). The decline extended over a large area of likely oyster habitat in Black Bay/Breton Sound ( $280$   $km^2$ ) and Barataria Bay ( $199$   $km^2$ ) and resulted in a total market equivalent loss of  $1.19 \times 10^9$  oysters across the freshwater impacted areas. Spat densities remained unchanged in

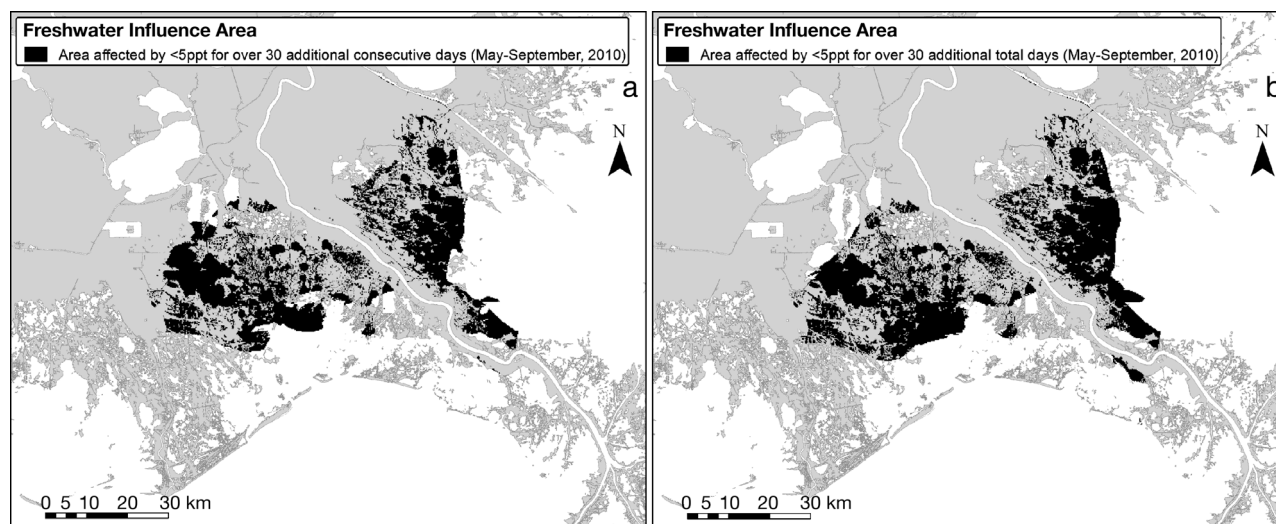


Fig. 7. Areas experiencing more than 30 additional days of salinities  $<5$  ppt from May to September 2010, compared to the average number of days  $<5$  ppt over the same period between 2006 and 2009. Areas of maximum freshwater influence estimated using (a) consecutive days of salinity  $<5$  ppt and (b) total days of salinity  $<5$  ppt

Black Bay/Breton Sound ( $0 \text{ m}^{-2}$ ). Seed densities increased slightly in Barataria Bay (Table 2). This small increase was considered as a case of 'no injury' and treated as a 0 in our injury calculations.

The Nestier tray/NRDA method also showed clear declines in oysters across all size classes. Predicted post-spill densities were lower for each size class (except oyster spat in Black Bay/Breton Sound, which were 0 before and after). The largest losses were seen in seed-sized oysters in both basins (Table 2), although decreases in market-sized individuals were also notable. These density declines would be expected to occur across large areas in Barataria Bay ( $766 \text{ km}^2$ ) and Black Bay/Breton Sound ( $591 \text{ km}^2$ ) based on the 5 ppt freshwater injury polygon. Unlike the previous injury estimates, we were able to create more spatially explicit injury estimates in each of the known and likely oyster habitats by predicting survival based on estimated salinities in each  $200 \text{ m}^2$  grid. This approach yielded a loss of  $2.78 \times 10^9$  market equivalent oysters.

## DISCUSSION

Nearshore ecosystems often act as repositories for contaminants released into the open ocean and rivers. Key to mitigating the ecosystem damage caused by the deposition of oil and other contaminants is often a swift response. In many cases, post-spill assessments of injury have indicated large impacts from the response activities (e.g. high pressure washing of the rocky intertidal during the 'Exxon Valdez' oil spill cleanup, see Driskell et al. 2001). The geographic extent (1000s of km of potentially impacted beaches and marshes) and duration (87 d of oil release) of the DWH oil spill necessitated unprecedented response and clean-up activities. The responses included the widespread uses of vast quantities of dispersants at sea, digging and disposing of beach sands, burning of oil at sea and in the marsh, extensive flushing of marshes with estuarine water, and a host of novel large-scale manipulations of hydrography and geomorphology (Martínez et al. 2012, Park et al. 2014). The large-scale introduction of freshwater from diversion structures is a novel approach to mitigation and one that our analysis suggests should be more thoroughly examined for future oil spills.

All 3 estimates of injury indicated substantial losses (over 1 billion market equivalent-sized oysters) resulting from the late spring/summer release of large quantities of freshwater from diversion structures in 2010. Loss of oysters from the release of freshwater

and consequent decreases in salinity is not a new phenomenon; extensive literature has documented negative impacts of low salinities on oysters (Chatry et al. 1983, Melancon et al. 1998, Turner 2006, La Peyre et al. 2009, Soniat et al. 2013). The extent of the loss in 2010, however, was highly unusual in its magnitude, which is a direct result of the release of high volumes of freshwater at a time of high water temperatures (La Peyre et al. 2013) and the need to keep the structures open for such a long time given the unexpectedly long duration of the spill event.

Vast areas of both basins experienced prolonged low salinity that would be expected to affect oyster survival. Our comparison of the 2010 salinity data to the baseline period of 2006–2009 demonstrated a vast spatial extent (100s of  $\text{km}^2$ ) of unusually low-salinity waters resulting from the diversion discharges. The injury polygon used for the NRDA spatial analysis defined the area that would lead to loss of oysters due to salinities  $<5$  ppt for more than 30 consecutive days. This level is consistent with literature findings that extended periods of low salinities result in extensive high oyster mortality. In our study, this threshold is also supported by the extensive Nestier tray data collected by the State of Louisiana.

Although we chose a threshold of 5 ppt for consecutive days, most of the oligohaline and mesohaline areas were near 1–3 ppt for the vast majority of the summer as a result of the continuous high discharge from the diversion structures. Our results are likely conservative because our decision to estimate exposure based on consecutive days of low salinity likely underestimates the full area impacted by decreased salinities in 2010 versus the historical baseline. The areas in the total days polygons are somewhat larger and hence would give a larger injury estimate.

Of the 3 approaches used, the fisheries temporal method is the simplest. This approach compared the average of baseline densities (2006–2009) to the average after the spill in 2010 at fixed stations. Changes in density were assumed to be a result of the unique conditions in 2010. This approach yielded the highest injury estimate of  $3.2 \times 10^9$  market equivalent oysters lost. The before/after aspect of this analysis is used in many types of environmental assessment (see Schmitt & Osenberg 1996) and controls for spatial variability by holding sites fixed over time. The limitation of the design is the lack of control or reference areas to contrast to the area of impact. Restricting the fisheries temporal approach to the area within the 5 ppt freshwater impact area decreased the injury estimate by over 60% ( $3.2$  vs.  $1.1 \times 10^9$  market equivalent oysters). This substantial decrease in the injury estimate prob-

ably represents a separation of the zone of complete mortality from extreme low salinity from areas that were more marginally impacted. Evidence for this is the overall increase in market size oysters in Barataria Bay. Closer inspection of the site pattern revealed some locations where larger oysters were seemingly unaffected by the extreme low-salinity areas. Such areas may have experienced periodic refuges of salt water because of their closer proximity to inlets or deep-water channels.

Our second approach to injury assessment, the NRDA spatial analysis, benefits from a design that delineates reference and impact sites in the same year. Sampling areas inside the polygon experiencing 5 ppt for >30 consecutive days served as the impact sites and those outside the polygon (but within the affected basins) served as our references, allowing us to control for basin-wide effects in 2010. Decreases in densities were evident in all size classes of oysters, with the exception of seed-size oysters in Barataria Bay. Applying the change in density over the area of our impact polygon resulted in an injury estimate of  $1.2 \times 10^9$  market equivalent oysters lost. The magnitude of this estimate agrees with the fisheries temporal approach when restricted only to the 5 ppt exposure polygon. Despite differences in site selection criteria between the LDWF sampling, which serve as the basis for the fisheries temporal assessment, and the NRDA sampling, both sampling protocols used diver-collected quadrats, which likely increases the compatibility of the comparison between studies. The strength of the before/after contrast with the fisheries temporal assessment coupled with the impact/control nature of the NRDA spatial design results in a relatively robust assessment that produces remarkably similar injury estimates and serves as validation for what we conclude is the minimum injury estimate owing to the freshwater-diversion response actions.

The fisheries temporal and the NRDA spatial assessment injury approaches both have limited spatial resolution; the change in oyster density is applied to the entire injury domain. Although both estimates adjust the injury to account for differences in percent cover of oysters in the different strata (mapped oyster reef, leased areas, likely oyster habitat), a universal delta density is applied to all strata. The Nestier tray/NRDA approach offers a spatially explicit injury estimate by using stratum-specific density changes as well as stratum-specific oyster percent cover estimates. The approach also uses the dose-response curve generated from the LDWF Nestier tray study to predict pre-spill oyster densities.

While the results of the different approaches to estimating injury are consistent in direction and rough magnitude, uncertainty exists in all 3 approaches and confidence intervals over the different approaches. We believe that the range 1.1 to 3.2 billion oysters lost encompasses the range of uncertainty due to measurement and procedural errors. The estimate of  $2.7 \times 10^9$  oysters from the Nestier tray injury approach is likely the most rigorous of our estimates because of its spatially explicit nature and its use of a detailed exposure-response relationship based on studies of freshwater impacts on oysters in the same basins where the injury occurred.

The design of the NRDA subtidal oyster studies was intended to evaluate abundance of oysters throughout the area where oil was observed on shorelines and surface waters. While toxicity studies have demonstrated that exposure to oil in water from the DWH spill could also have potentially harmed oysters, confirmation of such exposure is limited. In addition, in statistical analyses attempting to relate oyster densities to NRDA-collected data on oiling (measured in terms of co-located sediment tPAH) and oil-on-water (days, frequencies, and presence/absence), distance to heavily oiled shorelines did not support a discernable association between exposure to oil and subtidal oyster densities.

Besides oil and freshwater, several other factors known to affect oyster populations were examined during our study and were found not to offer compelling alternative explanations for the overall oyster decline observed across these 3 analyses. Competing hypotheses such as harvest pressure would not apply to 2010, since oysters were not harvested in the 2 basins as a result of public health concerns following the oil spill. Both the prevalence and severity of oyster disease (dermo) and oyster predators (such as oyster drills *Thais haemostoma*) would be expected to be substantively reduced by the reduction in salinities; furthermore, extensive NRDA oyster sampling and disease analysis in 2010 and 2011 showed very low prevalence of infection throughout our study area (<2 on a scale of 1 to 5, where scores of 3 indicate mortality potential). Temperature was typically either constant over the period of interest or exhibited a very slight decline and contributed minimally to variation within and outside of areas of freshwater exposure; thus it is unlikely to confound our results. In addition, precipitation in 2010 did not appear to be abnormal, based on an analysis of records from the US Historical Climatology Network daily dataset; potential impacts from drought or from excessive rainfall lowering salinities in these basins are therefore unlikely.



Using a series of methods and multiple data sets, progressing from the simplest to more sophisticated approaches, we consistently found that post-spill densities were lower than both temporal and spatial baseline comparison density estimates. Results were robust to data sets and changes in approach. We are confident that the freshwater exposures from Louisiana diversions in 2010 produced substantial, widespread oyster mortality in both Barataria Bay and Black Bay/Breton Sound basins. Discharge records show an atypical pattern and unusually high magnitude of freshwater flow from Davis Pond and Caernarvon diversions in the late spring and summer 2010. The efficacy of releasing large quantities of freshwater in response to the approach of oil from offshore should be carefully examined given the injury to oysters that we documented in this study. Such an analysis should be holistic at the ecosystem level and include responses of other organisms and habitats within the estuary.

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# Ecosystem services are lost when facilitation between two ecosystem engineers is compromised by oil

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**ABSTRACT:** Nearshore marine ecosystems are among the most productive areas in the world. Unfortunately, these areas also receive pollutants released into oceanic and riverine waters. Six years following the *Deepwater Horizon* oil spill, the largest in US history, the complexity of ecological injuries in this system is just now being elucidated. Here, we describe a novel pathway of injury from oil spills by documenting how the loss of oysters near marsh edge as a direct result of shoreline oiling and clean-up activities can double rates of coastal erosion. As part of the natural resource damage assessment, we examined the impact of shoreline oiling on eastern oysters *Crassostrea virginica* near the marsh edge at 187 sites in Louisiana and Mississippi Sound in 2013. For marshes that experienced heavy oiling, oyster habitat was 77% less abundant than in areas where no oil was observed. Areas near marshes characterized by more moderate levels of oiling had 33% less oyster habitat than areas where no oil was observed. Similarly, the number of sites without any oyster habitat was higher in heavily and persistently oiled areas compared to areas where no oil was observed (56 vs. 24%). The consequences of this loss are substantial and include loss of essential fish habitat, reduced nutrient cycling, and decreased erosion buffering. For a subset of the sites where erosion rate was also measured between 2010 and 2013 (n = 79), shoreline loss was more than twice as high (2.1 vs. 0.9 m yr<sup>-1</sup>) in areas lacking oyster cover. Our findings provide evidence that loss of nearshore oyster habitat can disrupt the strong facilitation between oysters and marsh vegetation.

**KEY WORDS:** Marsh · Oysters · Facilitation · Natural resource damage assessment · Sustainability · *Crassostrea virginica* · *Deepwater Horizon*

## INTRODUCTION

Within marine and estuarine landscapes, transition areas between habitat types ('edges') have extremely high biological production, serve as key nursery habitats for juvenile fish and mobile invertebrates, and are hotspots of biogeochemical cycling (Beck et

al. 2001, Bologna & Heck 2002, Piehler & Smyth 2011). The high biological productivity of these systems results from their juxtaposition between the end of the terrestrial biome and beginning of the sea. Shallow water (<1 m depth) habitats adjacent to sandy shorelines and salt marshes are the most common habitat edges in estuaries of the northern Gulf of

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Mexico (GoM). These nearshore habitats also represent one of the more sensitive and imperiled systems in the world (Vitousek et al. 1997, Lotze et al. 2006, Halpern et al. 2007). Sea level rise, reduced sediment load of riverine systems as a result of upstream flood control measures, urban and agricultural development, and contamination threaten the existence of these habitats and the ecosystem services they provide to society (Vitousek et al. 1997, Syvitski et al. 2005). Because these areas account for 24% of total global ecosystem services (Costanza et al. 1997, 2014), the economic consequences of habitat losses can be staggering.

Unfortunately, nearshore habitats are often the final repository for contaminants released into oceans. Oil spills are one of the primary examples of this phenomenon, with sandy shores, rocky intertidal zones, seagrass meadows, and marsh edge habitats shown to accumulate oil transported by surface waters (e.g. *Ixtoc I*, Tunnell et al. 1981; 'Exxon Valdez,' Peterson et al. 2003; Persian Gulf War, Gundlach et al. 1993; see also NRC 2003). Oiling of these shorelines results in ecological injury via multiple pathways: (1) physical fouling and smothering of the sensitive plants and animals that serve as the foundational species for the habitat (Smith et al. 1984, Peterson et al. 2003); (2) toxicity of the oil to plants and animals (Roth & Baltz 2009), which may last for extended periods of time if oil accumulates in bottom sediments or on marsh terrace soils (Reddy et al. 2002); and (3) oiled areas are also often the site of intensive response activities, which may result in physical degradation of the habitat (Driskell et al. 2001).

Whereas the ecological injuries associated with oiling vegetated and rocky intertidal shorelines are well established, less well-known is the response of eastern oysters *Crassostrea virginica* that form emergent reefs or smaller hummocks near marsh edges (Fig. 1). Historically, this narrow band of oysters along the marsh edge has largely been overlooked by marine resource agencies in the GoM and most of the Atlantic States (except South Carolina) because of the limited fishery value of the resource (Dyer & Leard 1994). Oysters present in this zone are not con-

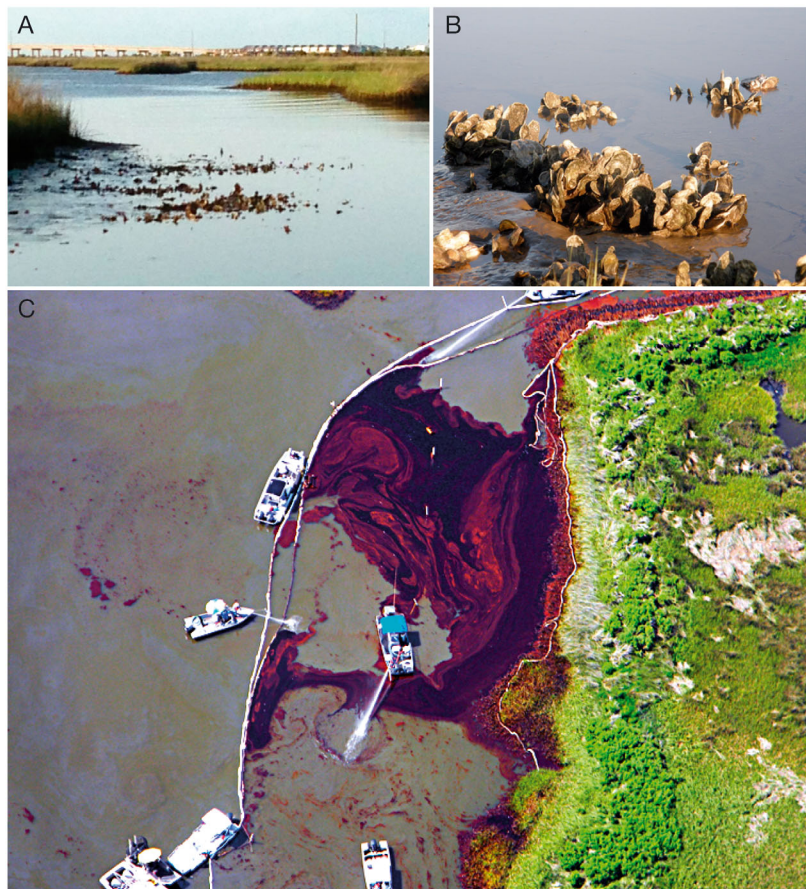


Fig. 1. (A) Nearshore intertidal eastern oysters *Crassostrea virginica*. (B) Typical oyster clump (hummock) along the shoreline. (C) Oiled shoreline

sidered desirable resources for the raw oyster market because of their irregular shape; they are difficult to harvest in the very shallow and soft sediment environments; and they often occur in areas closed to harvest because of water quality concerns (e.g. fecal coliforms, *Vibrio* spp.). The unattractiveness of these oysters for the fishery may increase their ecological importance, because in many areas these oysters serve as de facto sanctuary areas that form spawning stock reserves for oysters.

In contrast to their limited fishery value, the ecological importance of these oysters cannot be overstated. Nearshore oysters, like their subtidal counterparts, play an important ecological role through their filtration activities. Oysters remove sediments, phytoplankton, and detrital particles, potentially reducing turbidity and improving water quality (Dame & Patten 1981). The enhanced benthic-pelagic coupling that results from the suspension feeding of dense assemblages of bivalves can create hotspots of biogeochemical cycling (Piehler & Smyth

2011, Kellogg et al. 2013) within the estuarine landscape. The complex habitat formed by the gregarious settlement of oysters (reefs or hummocks) also provides critical refuge for benthic invertebrates as well as fishes and mobile crustaceans (Meyer & Townsend 2000, Peterson et al. 2003, Coen et al. 2007, Grabowski & Peterson 2007). The complex structure provided by oyster habitat may also facilitate (sensu Bruno et al. 2003) the maintenance and expansion of other habitats. Shallow subtidal and intertidal oyster reefs can facilitate emergent (saltmarsh, Meyer et al. 1997, Scyphers et al. 2011) and submerged vegetation (seagrass, Newell et al. 2002) in estuarine systems. For emergent shoreline vegetation like *Spartina patens* and *S. alterniflora*, nearshore oyster habitat may reduce wave energy that would normally result in shoreline erosion (Meyer et al. 1997, Piazza et al. 2005, Scyphers et al. 2011, NRC 2014, Lunt et al. 2017).

Recognizing both the importance of nearshore oyster habitat and the substantive degree of shoreline oiling that resulted from the *Deepwater Horizon* (DWH) oil spill in the north-central GoM, we designed a large-scale study that examined the distribution and fate of oyster habitat as a function of severity of shoreline oiling at 187 sites from Terrebonne Bay, Louisiana, through Mississippi Sound, Alabama (Fig. 2). For a subset of these sites ( $n = 79$ ), synoptic data on shoreline erosion was available from companion studies (non-persistent oil that normally presents as sheens), on the effect of the DWH oil spill on salt marshes (Hester et al. 2016, Willis et al. 2016) that allowed us to evaluate the consequences of changes in oyster cover on a critical ecosystem service—erosion control and facilitation of adjacent vegetated shorelines. Specifically, we tested the hypotheses that percent cover of oyster habitat and oyster abundance would change as a function of shoreline oiling resulting from the 2010 DWH oil spill and that loss of oyster habitat would result in changes in shoreline erosion rates.

## MATERIALS AND METHODS

Oyster habitat adjacent to emergent salt marsh (hereafter referred to as nearshore or intertidal oysters) was surveyed in the north-central GoM (between Terrebonne Bay, Louisiana, and Mississippi Sound, Alabama) to evaluate the condition (distribution and abundance) of nearshore oysters as a function of shoreline oiling or response activities, including releases of fresh water from large river diversion structures in Louisiana, following the DWH

oil spill. Sites (200 m long stretches of shoreline) were mapped to estimate oyster cover, as indicated by the presence of shell substrate. Where nearshore oysters were detected, sites were sampled for oyster density (number of oysters  $m^{-2}$ ) and size frequency. Sampling occurred between 14 February and 26 April 2013, i.e. 3 yr post spill.

### Site selection

Oyster sampling locations were selected from a large sampling universe of 2779 sites assessed in 2010 along the coastline of the northern GoM from Rollover Lake, Louisiana, to Apalachee Bay, Florida. Based on repeated observations by response surveys (shoreline cleanup and assessment technique, SCAT) and natural resource damage assessment (NRDA) teams, shorelines along the northern GoM were evaluated and assigned to 1 of 5 shoreline oil exposure classes, each describing a particular pattern of oiling over time (Nixon et al. 2016). For vegetated shorelines, these classes included 'heavy persistent oiling' (where heavy or moderate oiling was repeatedly observed over a period of  $\geq 12$  wk between April 2010 and February 2015), 'heavy/moderate oiling' (where moderate or heavy oiling persisted for  $< 12$  wk), 'lighter oiling' (non-persistent oil that normally presents as sheens), 'no oil observed,' and 'shoreline not surveyed' by linear shoreline evaluation methods. Of these sites, 187 along SCAT-surveyed shorelines were randomly chosen that represented the range of shoreline oiling categories and potential influence of the late spring/early summer freshwater diversion releases in 2010 from Terrebonne Bay, Louisiana, and Mississippi Sound, Alabama (Fig. 2). Many of these 187 sites were also chosen for evaluation under the NRDA-sponsored marsh edge and sandy shoreline study and the coastal vegetation study ([www.gulfspillrestoration.noaa.gov/oil-spill/gulf-spill-data/](http://www.gulfspillrestoration.noaa.gov/oil-spill/gulf-spill-data/)). Vegetation along the majority of sites was classified as mainland herbaceous saltmarsh (primarily *Spartina alterniflora*, *S. patens*, or *Juncus roemerianus*), with some sites classified as mixed black mangrove *Avicennia germinans* and *Spartina* spp. or *Phragmites australis* dominated. Additional locations from the original sampling universe were randomly added to represent and balance shoreline oiling categories and add coverage for Mississippi Sound, Breton Sound, or Marsh Island shorelines that could have been affected by freshwater releases. Freshwater diversion structures located in upper Barataria (Davis Pond) and Breton Sound



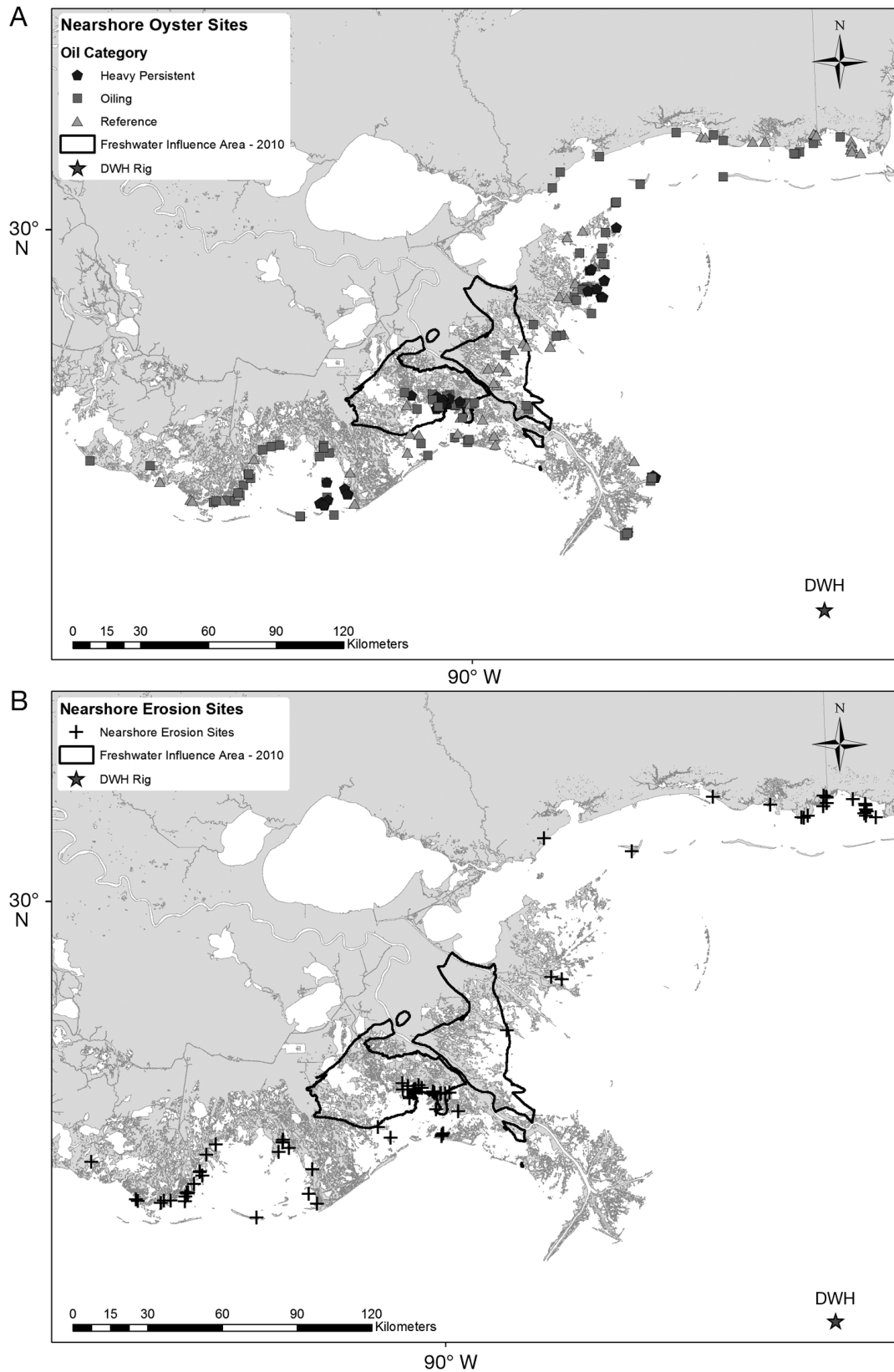


Fig. 2. North-central Gulf of Mexico, showing (A) locations of nearshore oyster sampling locations by oiling category and (B) locations where shoreline erosion rates were documented. DWH: *Deepwater Horizon*

(Caernarvon) Estuaries were open from late April through mid-August 2010 as a response action by the State of Louisiana to keep oil out of the estuaries (Martínez et al. 2012, Rose et al. 2014), releasing  $212\,000\text{ l s}^{-1}$  (Davis Pond) and  $226\,000\text{ l s}^{-1}$  (Caernarvon) of Mississippi River water directly into those estuaries. Normally these structures are opened only during the cooler winter and early spring months of each year because of potential impacts to oysters and other fisheries from low-salinity water exposure during the warm late-spring and summer months (Turner 2006, Rose et al. 2014). The additional sampling locations were probabilistically selected using the generalized random tessellation stratified sampling procedure (Stevens & Olsen 1999, 2004). The 187 sites sampled in 2013 represent a range of exposures to both oiling from the DWH spill in 2010 and changes in salinity resulting from actions undertaken by the State of Louisiana in response to the spill. Unsurveyed sites originally assigned to the 'not surveyed' oiling category were excluded from further consideration.

### Distribution of nearshore oysters

Following site selection, 4 field teams mapped oyster shell and other hard substrate over a total of 200 m of shoreline length at each of 187 sites. Each site was divided into 40 transects (20 on each side of a center location that was randomly chosen 0 to 5 m to the right of site center coordinates). Transects ran perpendicular to the shoreline, were 15 to 20 m in length (measured from the end of the vegetation line to offshore), and were spaced 5 m apart. At each sampling site, the transect start location (latitude and longitude) and direction (degrees) were recorded. Field teams cast a Y-shaped metal bar (secured to the end of a fiberglass meter tape) between 15 and 20 m from shore in a direction perpendicular to the shoreline and then slowly pulled it back along the sea floor, feeling for vibrations through the tape that would indicate the interaction of the bar with oyster shell. Transect lengths were measured beginning at the nearest meter mark on the tape. All field teams were trained in areas with known configurations of oyster shell and soft substrate prior to field work.

Substrate along each of the 40 transects at a site was recorded as either type 1 (soft mud), 2 (moderately firm mud, firm mud or sand, and buried shell), or 3 (exposed shell or reef) for each meter of the transect. Each meter of substrate was assessed either by the feel of the implement on the substrate as the bar

was pulled back toward the shore or through a combination of feel and visual observation when oysters were clearly visible. Some segments of the transects could not be mapped because the implement could not be thrown to the full 20 m extent, because of the presence of a dock or other obstruction, or if the field crew could not safely map the transect, for example, because of the presence of a deep channel extending from the shoreline at the transect start. The proportion of type 3 substrate cover, i.e. the percent cover of oyster habitat, for each mapped nearshore site was estimated as the total length of meters identified as type 3 substrate divided by the total length of meters mapped at that site.

We also examined the potential for response and oil clean-up activities that occurred on the shoreline to affect oyster cover. We reviewed records collected by NOAA related to shoreline oil spill response activities, including documents, database records, maps, and spatial data associated with pre- and post-oiling shoreline response activity operational work orders, and classified each site as receiving onsite response treatment or not treated. Onsite response activities included placement of booms adjacent to shorelines to prevent oil from reaching shorelines; flushing marsh surfaces with water; cutting and raking marsh vegetation; removing wrack and vegetation; raking heavy oil deposits from soil surfaces; and placing loose sorbent material (Zengel et al. 2015). We did not attempt to separate treated areas by severity of disturbance because all onsite response activities would be associated with physical alteration of the soft-sediment habitat seaward of the marsh, and most onsite response activities would involve landing boats on the marsh edge and deploying crews at the sites.

### Oyster abundance

Site mapping determined segments where oyster shell was found and therefore could be surveyed for abundance of oysters using quadrat sampling. Segments were defined as linear segments of exposed shell at least 1 m in length. Oyster abundance was sampled at up to 6 randomly selected quadrats per site. Quadrat sample locations were chosen from among segments where oyster shell was found using a 2-step process. First, each segment of exposed shell identified during mapping was numbered. Because segments could be multiple meters in length, field teams used a random number table to select 1 m long sections of segments for quadrat sampling. Up to 6

independent segments of exposed shell were sampled, depending on the number of segments identified and the length of available segments. In a given segment, each selected 1 m long section of shell was only sampled once. Quadrats of 1 m<sup>2</sup> were made of PVC. All substrate encompassed within the quadrat was collected by hand up to a depth of approximately 4 cm and placed in a labeled burlap sack. Samples were transported on ice to the Dauphin Island Sea Lab, Alabama. Within 48 to 72 h of collection, live oysters from each sample were enumerated in 3 size classes: market ( $\geq 75$  mm), seed (25–74.9 mm), and spat ( $< 25$  mm). Water quality measurements (salinity, temperature, dissolved oxygen) were also taken on the day of sample collection (recorded at the site center point at a water depth of at least 20 cm).

### Historical salinity

We examined the salinity history at each of our sites to determine whether average salinity differed by our design and could possibly confound our analyses. We utilized the network of salinity monitoring sites (both continuous sampling instruments as well as discrete samples publicly available) to determine if salinity varied by the 3 shoreline oiling categories and whether the sites were influenced by the 2 freshwater diversion areas. Salinity records were synthesized from several sources to complete the analysis: Louisiana Department of Wildlife and Fisheries, Louisiana Coastal Protection and Restoration Authority, Louisiana Department of Health and Hospitals, Louisiana Department of Environmental Quality, Mississippi Department of Environmental Quality, and US Geological Survey (complete details of the synthesis are given in Powers et al. 2017, this Theme Section).

### Erosion/shoreline change

Prior to sampling nearshore oysters, several other NRDA studies were undertaken to evaluate exposure and injury to nearshore flora and fauna. Seventy-nine nearshore oyster sampling stations were co-located with sites included in an evaluation of coastal wetland vegetation that collected synoptic data on shoreline erosion. The coastal wetland vegetation assessment (CWV) was intended to evaluate the effects of plant stem oiling on plant productivity, cover, and health and shoreline change. CWV sites were classified by degree of oiling on plant stems

and by vegetation type. At each site, a transect was established with 1 to 3 fixed-location, permanent plot pairs (for observations and destructive sampling). The length of the initial transect was determined by the length of oil penetration into the vegetation, as observed during the pre-assessment survey conducted in the summer of 2010, with a maximum length of 30 m from the intersection of water and vegetation. For reference sites, at which no oil was observed, the default transect length was 20 m. The number of vegetation sampling plots (up to 3) and the location of the plots along the transect were determined by transect length. The permanent location of the most shoreward plot pair was established with the shore edge of the plots located 1 m from the marsh edge at the time of the first sampling event.

In addition to the plant metrics collected at each plot, observations and measurements of shoreline change were made during each CWV sampling event. The length of the transect was first recorded when sites were established in the fall of 2010 (Louisiana sites) or the spring of 2011 (Mississippi and Alabama sites). At each subsequent survey (Spring 2011, Fall 2011, Fall 2012, Fall 2013), the distance from the inland stake to the marsh edge was measured, and observations of erosion or shoreline change were recorded. GPS coordinates were obtained from the shoreline and inland ends of the transect as well as the lower left corner of each plot (facing inland, the left-hand shoreward corner). Coordinates were generally obtained once per site with a GPS device with sub-meter accuracy (e.g. Trimble GeoXH), typically the first time a site was sampled. Each subsequent time a site was visited, a GPS device such as the Garmin GPSMAP 76 or the Garmin GPSMAP 60 was used (with an estimated accuracy of 3 m). The 79 oyster sites that are co-located with the coastal vegetation sites are used here to evaluate relationships between oyster cover and shoreline change from the fall of 2010 to the fall of 2013.

### Wind/wave energy

To examine other factors that could influence oyster cover and shoreline change, an exceedance wind frequency-based exposure index ( $EI_e$ ) was calculated and applied to sample stations. The index estimates exposure to extreme wind-driven wave energy based on fetch (in this work, the fetch at any given location on the shoreline is defined as the maximum over-water distance in a given direction from that point) and the proportion of all winds that exceed a specific

velocity in each evaluated direction. The index method was based on Keddy (1982) with modifications. The  $EI_e$  used in this analysis is based on critical wind speed exceedances using 2010–2013 data from 4 NOAA National Data Buoy Center stations between Lake Calcasieu, Louisiana, and Apalachicola Bay, Florida, resulting in a range of index values from 4 to 7593 for 187 investigated sites. Index values are computed for each year between 2010 and 2013 from overwater modified effective fetch values ( $f_i$ ), percent of wind speed observations exceeding a threshold using 8 directional bins, interpolated from 4 station data according to the following equation:

$$EI_e = \sum_{i=1}^8 e_i \times f_i \quad (1)$$

where  $i$  is 1 of 8 cardinal directional headings representing 45° intervals,  $e_i$  is the fractional proportion of time the wind was observed from the  $i^{\text{th}}$  direction at greater than the overall 20% exceedance value for all wind speed observations at that station in the given year, and  $f_i$  is the open water fetch in meters from the  $i^{\text{th}}$  direction. These indices were calculated for each of the 4 stations. After generating the index using metrics for each station, the 4 resultant wind rasters were averaged together at the location of each site using a weighting scheme based on the squared inverse distance from each respective station to derive a year-specific  $EI_e$  estimate. The overall 2010–2013  $EI_e$  used in the analysis is then calculated as the sum of the 4 computed annual  $EI_e$  indices.

### Data analysis

The sampling design for the analysis of percent cover of oyster habitat represents a 3 × 2 factorial ANOVA evaluating 3 shoreline oiling categories and 2 freshwater conditions (inside or outside a polygon of freshwater influence) (Table 1). Freshwater influence polygons were drawn based on examination of the duration and timing of freshwater releases in 2010 compared to typical and historical freshwater

flows (Fig. 2). For the purposes of evaluating near-shore oysters, we reduced the 5 shoreline oiling categories to 3: heavy persistent oiling as defined above, oiled, and no oil observed. The heavy/moderate and lighter oiling categories were combined into an ‘oiled’ category to distinguish effects of heavy persistent oiling, such as heavy fouling and smothering, from those sites that experienced more subtle effects of oiling (e.g. less physical fouling). Dependent variables in the analyses included percent cover of nearshore oyster habitat and density of market-, seed-, and spat-sized oysters per site. For each site, the abundance of oysters by size categories was averaged for the analyses using up to 6 replicate quadrats. Sites with and without any oyster cover were included in the analysis of percent cover, whereas only sites where quadrats were collected (positive percent cover) were used in the analyses of abundance.

The effect of onsite shoreline response/oil clean-up activities on percent cover of oyster habitat was tested using an unpaired, 2-tailed  $t$ -test assuming unequal variances. Treated sites were compared to untreated sites in the heavy persistent and oiling categories (as no response activities occurred in the no oil observed sites). We pooled treated and untreated sites across the 2 oiling categories to provide sufficient replication for the test. Although the potential for an interaction between treatment and oiling category exists, the lack of response activities in any of the no oil observed sites prevents resolving any interaction. Because of the potential bias of including all untreated sites from the no oil observed areas, which had higher percent cover than the other oiling categories, we excluded all no oil observed sites from this analysis.

To determine if the presence of oyster cover affected the erosion rate of adjacent vegetated marsh, we performed a series of univariate analyses. First, we tested whether the presence/absence of oyster habitat as measured in the winter of 2013 is associated with lower shoreline erosion from the fall of 2010 to the fall of 2013 using an unpaired, 1-tailed  $t$ -test assuming unequal variance. Next, we examined site-specific values of  $EI_e$  scores to evaluate the possibility that areas with higher erosion potential had a higher probability of not having oyster cover. To determine whether erosion potential  $EI_e$  score differed between areas with and without oyster cover, we

Table 1. Distribution of sampling sites by shoreline oiling and freshwater (FW) diversion treatment category. Numbers in parentheses indicate sites where onsite response activities occurred

FW influence	Reference	Shoreline oiling		Total
		Oiled	Heavy persistent	
Outside polygon of FW influence	34 (0)	63 (34)	24 (22)	121
Inside polygon of FW influence	20 (0)	31 (11)	15 (12)	66
Total	54	94	39	187

performed a 2-tailed *t*-test analyzing whether the sum of annual site  $EI_e$  values from 2010–2013 differed in areas where oysters were present versus absent. Presence was defined as sites with  $\geq 0.5\%$  cover of oyster habitat. All analyses were performed in JMP version 11 (SAS Institute) on untransformed data. Mean values are given  $\pm$ SE.

## RESULTS

### Distribution of oyster habitat

Percent cover of oyster habitat varied as a function of both shoreline oil exposure and location of sampling within the freshwater diversion release polygons. The interaction between the 2 factors was not significant (Table 2). Lowest percent cover values were recorded in areas adjacent to marshes that experienced heavy and persistent oiling ( $2.3 \pm 0.7\%$ ), followed by areas that experienced more moderate and less persistent oiling ( $6.9 \pm 1.3\%$ ) and reference shorelines where no oil was observed ( $10.3 \pm 2.1\%$ ; Fig. 3). The proportion of sites with no oysters, i.e. sites with percent cover of oyster habitat  $< 0.5\%$ , was also highest adjacent to marshes that experienced heavy persistent oiling (56%), followed by oiling (43%) and no oil observed (24%). Sampling locations within the freshwater diversion-affected areas had higher percent cover of oyster habitat than areas outside ( $9.6 \pm 2.0\%$  vs.  $5.4 \pm 0.8\%$ ). Onsite response activities affected percent cover of oyster habitat. For

oiled sites with documented onsite response activities, percent cover was significantly lower than oiled areas that did not have cleanup or response activities (*t*-test assuming unequal variances,  $t = -3.20$ ,  $df = 179$ ,  $p = 0.002$ , 2-tailed). The mean oyster percent cover at treated sites was 3.8% compared to 7.9% at untreated sites (Fig. 4).

Live oysters were found at virtually all sites that had oyster habitat. In most cases, abundance of oysters did not vary significantly by shoreline oiling category, sampling location relative to a freshwater diversion polygon, or their interaction (Table 2). The interaction between the 2 factors, i.e. shoreline oiling and freshwater diversion polygon, was not significant in any of the ANOVAs. Abundance of spat-, seed-, or market-sized oysters in areas where oyster habitat was present did not differ significantly with oiling, although a trend of lower abundances for spat- and seed-sized oysters was noticeable in the heavy persistent oiling category (Table 2, Fig. 5). The only exception was the abundance of market-sized oysters, which was significantly higher in locations that were within the freshwater diversion polygon ( $4.7 \pm 1.5$  vs.  $2.5 \pm 0.8$  oyster  $m^{-2}$ ).

The historical salinity pattern revealed a consistent difference between sites within and outside the freshwater diversion influence area but not among shoreline oiling category within those zones (Fig. 6). Salinity for reference, oiled and heavy persistent oiled sites ranged from 7–18 ppt inside the area influenced by the freshwater diversion openings and 18–24 ppt in the area outside the freshwater diversion.

Table 2. Summary of 2-way ANOVAs testing the effects of shoreline oiling category and potential exposure to freshwater diversion in May to August 2010 on oyster cover and oyster density metrics measured in February 2013. Significant effects ( $p < 0.05$ ) are presented in **bold**

Response	Sample size	Source	df	SS	F	p
Oyster habitat (% cover)	187	Shoreline oiling category	2	0.18	6.24	<b>0.002</b>
		Freshwater diversion exposure	1	0.07	4.74	<b>0.031</b>
		Interaction	2	0.06	1.95	0.145
		Error	181	2.60		
Market-sized oysters (no. $m^{-2}$ )	119	Shoreline oiling category	2	2.46	0.03	0.976
		Freshwater diversion exposure	1	238.20	4.76	<b>0.031</b>
		Interaction	2	203.59	2.03	0.136
		Error	113	5654.96		
Seed-sized oysters (no. $m^{-2}$ )	119	Shoreline oiling category	2	3114.65	1.27	0.285
		Freshwater diversion exposure	1	1914.91	1.56	0.214
		Interaction	2	1319.62	0.54	0.586
		Error	113	138791.06		
Spat-sized oysters (no. $m^{-2}$ )	119	Shoreline oiling category	2	274.79	1.10	0.338
		Freshwater diversion exposure	1	24.01	0.19	0.663
		Interaction	2	14.04	0.06	0.946
		Error	113	14180.49		



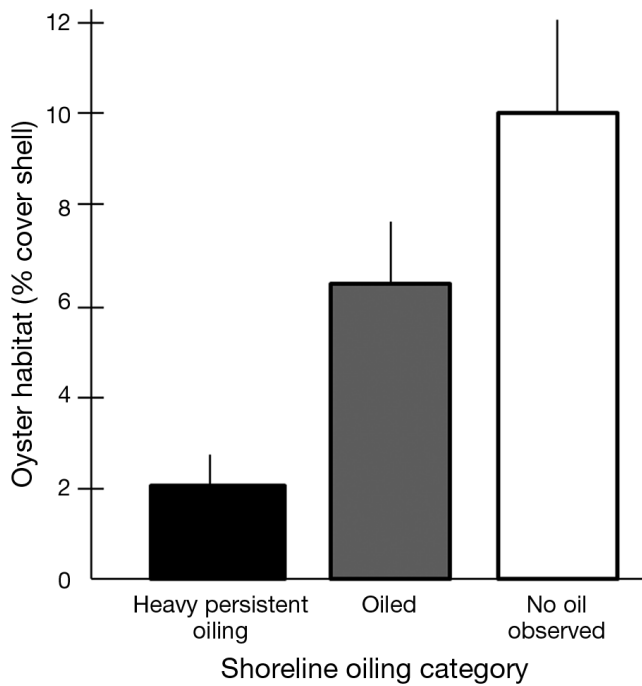


Fig. 3. Mean ( $\pm$ SE) percent of oyster habitat found adjacent to vegetated shorelines with heavy persistent, moderate (oiled), and no oiling from Terrebonne Bay, Louisiana, to Mississippi Sound, Alabama

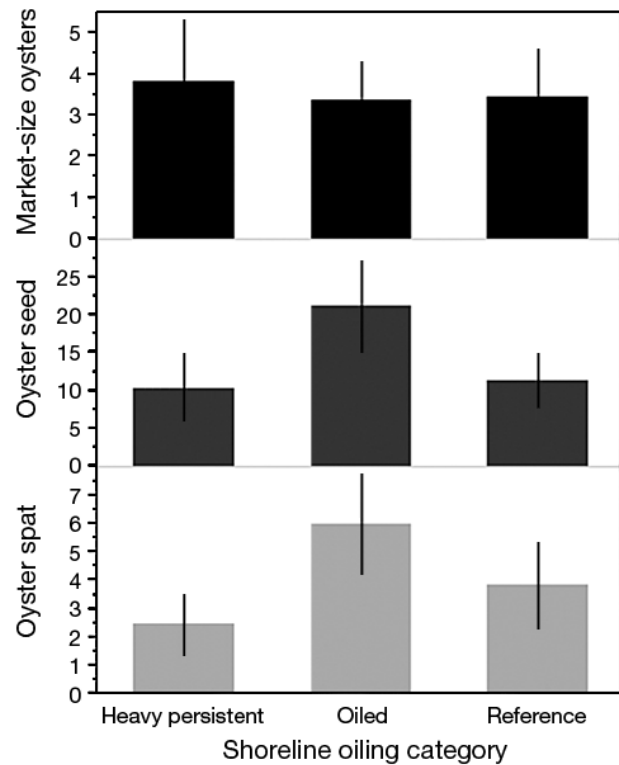


Fig. 5. Mean ( $\pm$ SE) density (oysters  $m^{-2}$ ) by shoreline oiling exposure category measured in Winter 2013 at 187 sites from Terrebonne Bay, Louisiana, to Mississippi Sound, Alabama, for market-, seed-, and spat-sized oysters

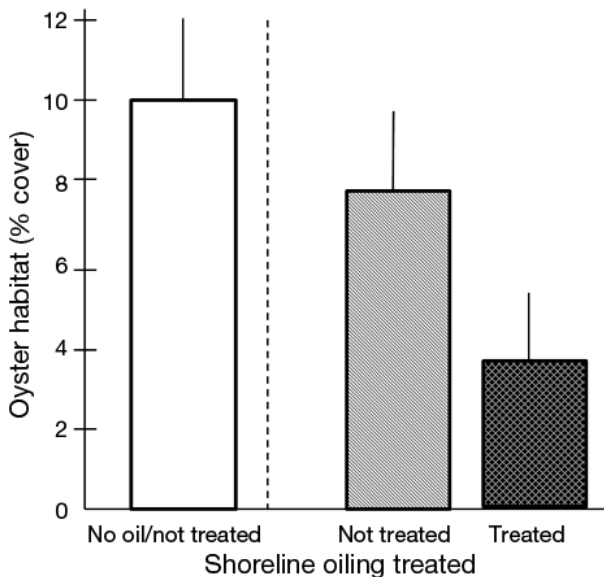


Fig. 4. Mean ( $\pm$ SE) percent cover of oyster habitat in heavy persistent oiling and oiled areas subject to response and clean-up activities (Treated) and where no treatment occurred (Not treated). Means are significantly different at  $p < 0.05$ . Mean percent cover for untreated/no oil observed sites (white bar) is shown for reference only and was not included in the statistical analysis

### Erosion and percent cover

Erosion of marsh occurred at almost all of the 79 sites where oyster metrics and shoreline change (transect lengths) were measured. The presence of oyster habitat was associated with significantly reduced shoreline erosion in the adjacent marsh ( $t$ -test assuming unequal variances,  $t = -1.83$ ,  $df = 27$ ,  $p = 0.0396$ , 1-tailed). Where oyster cover was absent, erosion was  $8.4 \pm 2.5$  m over the 3 yr period. In contrast, erosion rate where oyster habitat was present was  $3.8 \pm 0.6$  m over the same period. While oyster presence/absence significantly affected marsh erosion, onsite response activity along the shoreline did not have a significant effect on erosion rate ( $p = 0.152$  for  $t$ -test) although a trend of higher erosion at sites with onsite response activities was noted (mean erosion  $7.2 \pm 1.6$  at treated sites vs.  $4.6 \pm 1.5$  m over the study period). Linear correlations between percent cover and erosion over the 3 yr window failed to produce a significant relationship ( $p = 0.693$ ); however, higher rates of erosion were generally associated with lower percent cover. Finally,  $EL_e$  scores did

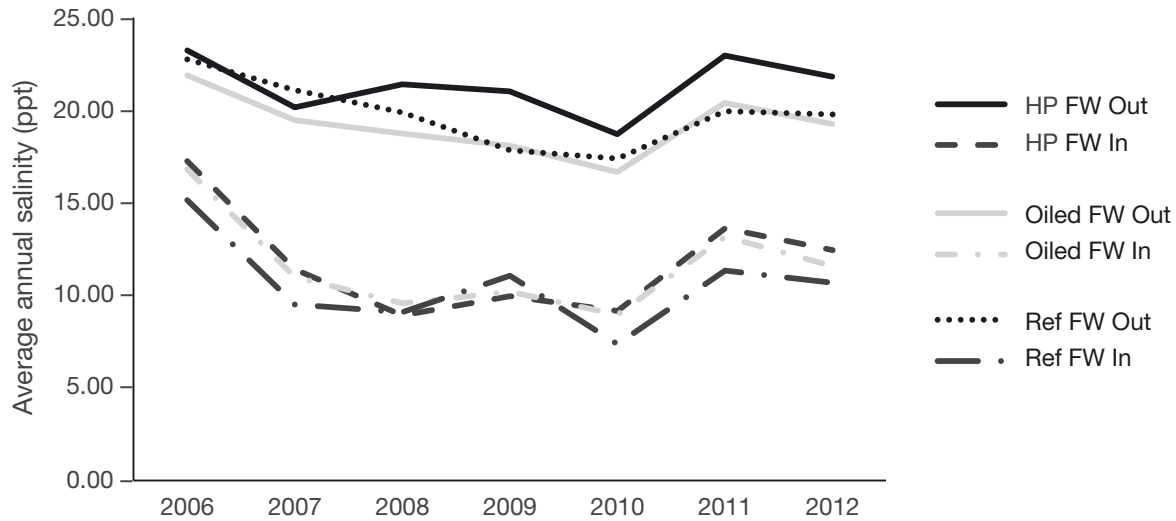


Fig. 6. Average annual salinity (ppt) of oyster sampling sites by shoreline oiling category (heavy persistent [HP], oiled, reference [Ref]) and freshwater diversion exposure (FW In, FW Out: inside, outside of freshwater diversion influence area) from 2006 to 2012

not differ in areas with and without oyster habitat ( $p = 0.820$ ). Oyster habitat occurred over the full range of  $EL_e$  scores with mean values similar between areas with ( $5072 \pm 595$ ) and without oyster habitat ( $5280 \pm 704$ ).

## DISCUSSION

The prevalence of biogenic habitat in nearshore areas of the world is a key factor that explains the high productivity of these areas. Biogenic habitats are hotspots of primary and secondary productivity within the coastal landscape by providing refuge and food for juvenile fish and invertebrates and enhanced nutrient cycling, among other ecosystem services (Heck et al. 2003). In the GoM, the most common habitat encountered in estuarine and coastal areas is salt marsh (Minello et al. 2003). The frequent inundation of marshes, driven primarily by wind in this microtidal environment, provides access to food and shelter for marine animals and accounts for a large secondary production of invertebrates (Rozas 1995, Minello et al. 2003, Haas et al. 2004). Oyster reefs and seagrass meadows, while less common, also provide shelter and food for fish and invertebrates (Heck et al. 2003, Coen et al. 2007, Grabowski & Peterson 2007). Often these habitats occur adjacent to one another and form a complex mosaic of structural refuge and foraging habitat for fish and invertebrates as well as terrestrial and avian vertebrates (Grabowski et al. 2005). One of the most common couplings along the US Atlantic and Gulf Coast is that of saltmarsh and fringing oyster habitat (Grabowski et

al. 2005, Geraldi et al. 2009). Our estimate that 76% of salt marsh habitat in the no oil observed areas had adjacent oyster cover shows that such pairing frequently occurs in the northern GoM. The average percent cover of oysters in the no oil observed was 10%, which indicates that this landscape feature is sufficiently abundant to be important for ecosystem processes in the area (e.g. fish utilization and nutrient cycling). Baseline information on fringing oyster habitat is largely absent from the published literature, thus our estimate that the habitat is ubiquitous (76% of unimpacted sites) and relatively abundant within sites (10% cover) establishes a new baseline to examine the importance of this habitat coupling in coastal ecosystems.

As with other oil spills, oil transported in surface waters following the explosion and blowout of the DWH well was deposited in vast quantities along vegetated and non-vegetated shorelines. While oiling and recovery of salt marshes has received substantive attention in previous oil spills, no published studies exist on the fate of nearshore oyster habitat following an oil spill. Reduction in the amount of oyster habitat was evident in areas that were classified as having experienced shoreline oiling compared to areas where no oil was observed. For shorelines that were characterized as heavily and persistently oiled, shell habitat that would support oysters was on average only 2% compared to over 10% in non-oiled areas. In our study that surveyed an area of roughly 4000 m<sup>2</sup> (200 m shoreline length  $\times$  20 m width), this change in percent cover is equivalent to losing approximately 320 m<sup>2</sup> of oyster habitat at each of those sites characterized as heavy persistent oiling and

approximately 135 m<sup>2</sup> in areas characterized as oiled. Oyster habitat appeared similar among sites where it was detected, with live oyster in all 3 oyster size classes present at comparable densities across the oiling treatments. The 2 patterns (percent cover decline while remaining reef had similar density) suggest that the injury resulting from the DWH oil spill in summer of 2010 was largely a function of an acute disturbance that occurred during or within 1 yr after the oil spill (assuming approximately 2 yr for oyster growth from spat to market size; see growth function in Soniat et al. 2012).

The pattern of reduced oyster cover could be a function of extensive oiling and smothering of oysters along the shoreline during the spill as well as disturbance from shoreline cleanup and onsite response activities that occurred in oiled areas. The latter is supported by the pattern of reduced percent cover of oyster habitat in areas where response activities occurred. Deployment of field crews, landing of vessels, booms scouring the shallow bottom sediments, flushing of oil-soaked contaminated marshes with water all could result in trampling, smothering, and burial of oyster habitat. The difference between untreated and treated shorelines does not preclude that direct oiling of oyster habitat also contributed to the loss of oyster cover. Oysters that occur near the marsh edge often inhabit very muddy, soft sediments, and their position above the sediment surface is a precarious one because the weight of the oyster itself would be expected to cause sinking over time. The filtration activity of the oysters and the annual recruitment of new oysters that gregariously settle on oyster shell may thwart burial and promote vertical relief. Because most of the shell habitat has a vertical relief of no more than 50 cm (S. Powers pers. obs.), smothering by the mousse-like oil residue that coated many shorelines (Fig. 1) following the oil spill could have prevented filtration and additional recruitment. Over a relatively short time this degraded oyster habitat could have been buried.

Regardless of the injury pathway (shoreline oil spill response activities or direct oiling of shorelines), oyster habitat in areas receiving oil following the DWH oil spill was severely degraded. Given the documented decreases in oyster habitat over recent decades in the GoM (see zu Ermgassen et al. 2012), such episodic losses are a reason for added concern and could further stress a habitat near its tipping point. Because the fishery value of nearshore oysters in the northern GoM is limited (although these areas have the potential to serve as a de facto spawning sanctuary that 'seeds' areas of harvest), the loss of

ecosystem functions and services is the pressing concern for resource managers. Extensive literature now exists on the ecological benefits of oyster reefs (see Coen et al. 2007, Grabowski et al. 2012 for recent reviews) and supports that the loss of habitat can have effects on nutrient cycling (Piehler & Smyth 2011, Beseres Pollack et al. 2013, Kellogg et al. 2013), fish and invertebrate production (Peterson et al. 2003), water clarity (Newell & Koch 2004, Grizzle et al. 2008), and shoreline stabilization (Scyphers et al. 2011). Collectively, the annual economic value of these services (excluding oyster harvest) was recently estimated to total US \$5500–99000 ha<sup>-1</sup> (Grabowski et al. 2012). We were able to test the potential for one of these ecosystem services, viz. shoreline stabilization, within our study area by coupling our measurements of oyster habitat with measurements of shoreline erosion collected under companion studies (Hester et al. 2016, Willis et al. 2016).

The presence of oysters along the shoreline reduced the 3 yr erosion rate by over 50%. Our analysis of  $EL_e$  scores also provides strong evidence that the pattern is not a function of oyster preference or physical disturbance in areas that experience more erosive forces. Oyster cover occurred across a spectrum of erosive conditions as measured by  $EL_e$  scores, thus oyster cover is not restricted to only low energy sites where erosion would be expected to be reduced. Consistent through all of our findings is the role of oyster habitat reducing erosion. Although shoreline stabilization has been increasingly cited as a potential benefit of oyster reefs, few empirical studies exist that quantify the benefit (NRC 2014, Powers & Boyer 2014). Our study is the first to provide field-collected data over a large geographic area that establishes that the presence of oyster reefs mitigates or buffers erosion. Previous studies (e.g. Piazza et al. 2005, Scyphers et al. 2011) were performed at sites spanning just a few 100s of m. Piazza et al. (2005) demonstrated that spreading low-relief oyster shell cultch could reduce shoreline retreat in areas of low to moderate erosive energies in Sister Lake, Louisiana. Scyphers et al. (2011) demonstrated that erosion and vegetation retreat was reduced behind restored oyster reefs near Point aux Pins, Alabama. Interestingly, both studies demonstrated effects of similar magnitude: 40% reduction in Scyphers et al. (2011) and 25% in Piazza et al. (2005). Emergent oyster habitat protects shorelines by dampening wave energies and potentially trapping sediments eroded from the shoreline or transported through nearshore currents (NRC 2014). The lack of a strong correlation between

percent cover and erosion (our finding is based on presence/absence) demonstrates the complexity of the relationship. Our percent cover estimate is based on site-level measurements over an area of 4000 m<sup>2</sup> extending 20 m from the shoreline. Sites varied in their distribution of oyster habitat, but in general, oyster cover was greatest closer to the shoreline (peaking around 2–3 m from the marsh edge). The local arrangement of oyster patches likely influences the wave attenuation properties of oyster habitat (Lunt et al. 2017), and this unexplained variance could contribute to the lack of a strong fit in the relationship between oyster density and erosion (Koch et al. 2009).

Our results demonstrate that nearshore marsh ecosystems were degraded through both direct (loss of oyster habitat) and indirect (erosion of marsh from loss of oyster habitat) pathways as a consequence of the DWH oil spill. Reduction in oyster habitat occurred as a direct result of shoreline oiling or onsite response and oil cleanup activities. This reduction leads to increased marsh erosion rates. While indirect effects of oil spills have been documented (see Peterson et al. 2003), the disruption of the strong facilitation between 2 ecosystem engineers represents a previously undocumented pathway of injury resulting from an oil spill. Unfortunately, additional monitoring necessary to document recovery time was not conducted due to cost considerations; however, natural recovery times would be expected to take decades given modest natural spreading. Rodriguez et al. (2014) reported natural spreading rates of 10–30 cm yr<sup>-1</sup> of radial expansion for restored oyster reefs in North Carolina with high vertical relief and shell material, but extremely low (<5 cm) on patches without adequate hard substrate for oysters to recruit. The latter reef types are more similar to those measured in our study. Consequently, the lost expanses of oyster habitat along GoM shorelines will persist until intervention (shell placement as part of a restoration program) is initiated. This lingering effect would be expected to result in further loss of vegetated marsh and exacerbate declines in ecosystem function. Given the current high rate of wetland loss in the northern GoM (Kennish 2001), particularly in Louisiana where much of our study was conducted, and the decades-long, slow demise of oyster reefs in the GoM (zu Ermgassen et al. 2012), the additional loss of both habitats resulting from the DWH oil spill represents a conservation emergency that requires rapid restoration of this habitat mosaic.

The findings of this study, based on co-located coastal wetland vegetation study sites and oyster

study sites, do not preclude other findings of relationships among shoreline vegetation, erosion, and/or oil exposure based on further analysis of data from the universe of the nearshore sites studied as part of the DWH NRDA. Moreover, the loss of vegetated shoreline as a result of oyster habitat degradation does not preclude other direct and indirect effects resulting from the oiling of vegetated areas, which our study had limited statistical power to test. Instead, this study documents an entirely unreported pathway of injury from oil to greater potential marsh erosion.

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# Oil spill response-related injuries on sand beaches: when shoreline treatment extends the impacts beyond the oil

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**ABSTRACT:** Studies of oil spills on sand beaches have focused traditionally on the effects of short-term oil exposure, with recovery of sand beach macrobenthic communities occurring within several weeks to several years. The *Deepwater Horizon* spill resulted in chronic, multi-year re-oiling and up to 4 yr of extensive and often intensive treatments. Of the 965 km of sand beaches that were oiled, shoreline treatment was documented on 683 km. Intensive mechanical treatment was conducted from 9 to 45 mo after the initial oiling on 32.4 km of shoreline in Louisiana, and deep beach excavation/sifting and tilling was conducted along 60.5 km in Louisiana, Alabama, and Florida, often along contiguous lengths of beach. Recovery of sand beach invertebrate communities from the combined effects of oiling and treatment would likely be delayed by 2 to 6 yr after the last response action was completed. We introduce the concept of 'Response Injury' categories that reflect both intensity and frequency of beach treatment methods. We use the literature on similar types of disturbances to sand beach communities (foot traffic, vehicular traffic, wrack removal, beach nourishment) to describe the expected impacts. Temporal patterns of response-related disturbances can affect seasonal recruitment of organisms and the overall rate of ecosystem recovery from both oil exposure and treatment disturbance. This concept provides a framework for specifically assessing response-related impacts in future spills, which has not been considered in previous injury assessments.

**KEY WORDS:** *Deepwater Horizon* · Oil spill · Sand beach · Shoreline treatment · Shoreline cleanup · Impact · Recovery

## INTRODUCTION

Approximately 70% of the land–sea margin globally, and the predominate portion of the land–sea margin in the northern Gulf of Mexico, consist of exposed sand beaches (Rakocinski et al. 1991, Dugan et al. 2010). This generally narrow, but ubiquitous landform supports a diverse, but cryptic, biological community that, along with the physical structure of the beach, provides an array of ecosystem services, some of which are widely recognized and exploited commercially, some of which are not. The list of recognized

services provided by sand beaches includes: (1) sediment storage and transport; (2) wave dissipation and buffering during storms; (3) scenic vistas and recreation; (4) groundwater filtration; (5) nutrient mineralization and recycling; (6) maintenance of biodiversity and genetic resources; (7) carbon transfer from primary producers and decomposers to species of broad public interest, such as birds and fish; and (8) functional links between terrestrial and marine environments (Defeo et al. 2009). Many of these services can be altered, diminished, or destroyed by oiling of the beach and shoreline cleanup actions to remove the oil.

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The *Deepwater Horizon* (DWH) oil spill resulted in the oiling of 965 km of sand beach habitat from Florida to Texas, USA (Fig. 1; Nixon et al. 2016). Oil first began stranding in May 2010, which was soon followed by shoreline cleanups. Oil stranded in discontinuous waves over a 3 mo period, resulting in the incorporation of oil into the sediments in supratidal, intertidal, and nearshore subtidal habitats, at depths that could be greater than 1 m (Michel et al. 2013). Over time, these oiled sediments became remobilized, broken into oil:sediment balls, and subsequently were reburied as the beaches eroded and then accreted as part of the normal beach cycle. Most of the initial oil stranded when the beaches were in an eroded state, following a series of strong winter cold fronts from the prior year. Gulf beaches erode during the approach of these winter cold fronts by a combination of strong north winds that move sand from the backshore to the upper beach face, followed by southerly winds that transport some of the sand into the subtidal nearshore once the fronts have moved through (Dingler et al. 1992). Subsequent accretion following these erosional events deeply buried some of the DWH oil, which persisted in spite of the passage of several hurricanes (Michel et al. 2013). Beach profile data collected by field teams documented this post-oiling beach accretion from Louisiana to Texas (Deepwater Horizon SCAT Program unpubl. data). Removal of buried and exposed oil required extensive and prolonged uses of mechanical and manual treatments to meet cleanup endpoints. Cleanup endpoints were defined by a maximum amount of oil from the DWH spill (MC-252 oil) that could remain in the beach habitat (Table 1). On many beaches managed by federal agencies as part

of national park lands or national wildlife refuges, the decision was made to have less stringent cleanup endpoints and forego intensive mechanical treatment.

Initially, most surface oil was removed using manual methods. However, by the fall of 2010, the Unified Command, composed of the US Coast Guard, BP, state, and local representatives responsible for directing the response, decided to use mechanical sifters to minimize sand removal, which was implemented mostly on amenity beaches in the 3 eastern states of Florida, Alabama, and Mississippi. The decision was made because of the large extent of shoreline to be cleaned, the deep burial of the oil, the patchy distribution of oiled sediments, the stringent cleanup endpoints for amenity beaches, and the push to open beaches in time for the 2011 tourist season. Mechanical sifting was conducted at night when the oiled sediments were cooler and less likely to break up as they passed through the screens. Night-time operations had the added potential impact to nocturnal fauna, such as sea turtles, ghost crabs, and wrack-associated species.

Another consequence of the deep burial of the oil was that most intensive removal operations were conducted in the supratidal zone. The large number of vehicles used during the response traveled mostly in the supratidal zone. Though efforts were made to restrict vehicles to certain corridors, incipient dune areas were heavily trafficked. Manual removal was conducted in all tidal zones. Many of the oiled sediments were referred to as surface residue balls, because they contained only about 10% oil but were cohesive. Long-reach tracked backhoes operated at the water line in the intertidal zone during efforts to remove submerged or buried oil:sediment mats from nearshore subtidal areas.

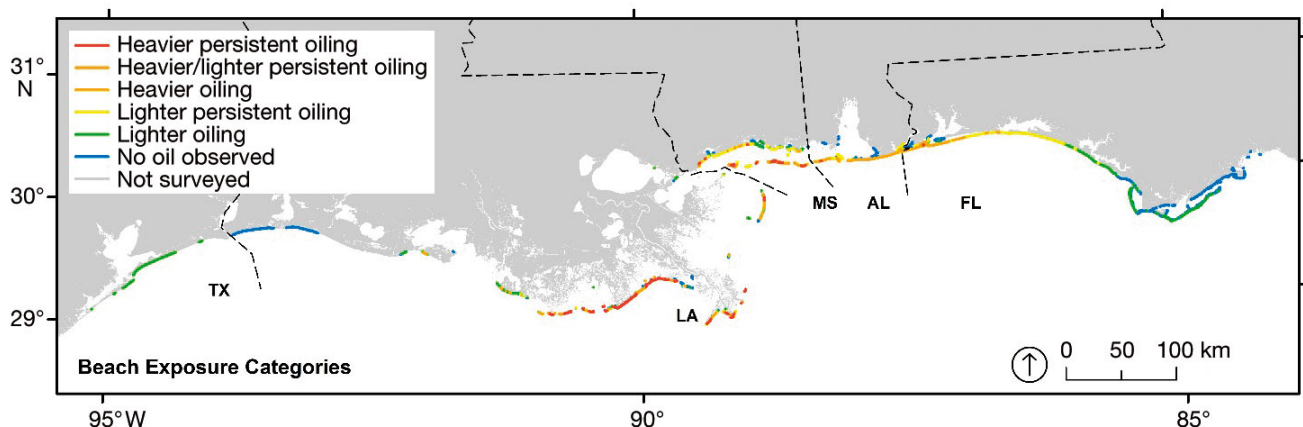


Fig. 1. Distribution of the maximum oiling category for all beaches affected by the *Deepwater Horizon* (DWH) oil spill. Compare with the maximum Response Injury (RI) in category per year (Fig. 6). See Nixon et al. (2016) for more detail on how oiling categories were defined and determined

Table 1. Cleanup endpoints for sand beaches for the *Deepwater Horizon* oil spill. SRB: surface residue ball

Shoreline type	Surface oil	Subsurface oil
<b>Eastern states: Florida, Alabama, Mississippi</b>		
Residential and amenity sand beaches	No visible MC-252 oil, or...*	No visible MC-252 oil, or...*
Non-residential or non-amenity sand beaches	<1% visible surface oil and oiled debris, and no SRBs >5 cm diameter, or...*	No subsurface oil exceeding 3 cm in thickness and patchy (<50%) distribution that is greater than oil residue, or...*
Beaches in special management areas (state and federal wildlife refuges, parks, wilderness areas)	Subject to direction of special area managers: <1% surface oil and oiled debris, and no SRBs >2.5 cm, or...*	Subject to direction of special area managers: no subsurface oil exceeding 3 cm in thickness and patchy (<50%) distribution that is greater than oil residue, or...*
<b>Louisiana</b>		
Residential beaches	No visible oil that is MC-252, or...*	No visible MC-252 subsurface oil above stain, or...*
Non-residential beaches and non-federal special management areas	<1% distribution of oil and oiled debris, or...*	No subsurface oil exceeding 2.54 cm in thickness and patchy (<50% distribution) that is greater than oil residue, or...*
Beaches in federal special management areas	<1% surface oil and oiled debris, or...*	No attempt to remove subsurface oil
*[or...] as low as reasonably practicable, considering the allowed treatment methods and net environmental benefit		

Most mechanical removal operations in Florida, Alabama, and Mississippi were terminated by March 2011; however, mechanical removal methods were intermittently implemented until 2013 for some shorelines in Alabama. Due to beach erosion concerns in Louisiana, mechanical methods were initially used only on 1 amenity beach. However, mechanical excavation methods were extensively used both in the intertidal and supratidal zones in Louisiana during 2011 to 2012, to remove deeply buried oil that had persisted on several beaches through several hurricanes. Mechanical augers on small tracked vehicles were used extensively to delineate buried oil for removal; for example, nearly 38 000 auger holes were excavated in a 10 × 10 m grid between November 2012 and August 2013 on several beaches in Louisiana. Manual removal methods continued at different frequencies for years; active shoreline treatment operations were deemed complete in Florida, Alabama, and Mississippi in June 2013 (lasting 3 yr), and in Louisiana in April 2014 (lasting nearly 4 yr).

As part of the natural resource damage assessment (NRDA) for the DWH spill, the impacts of oil exposure and subsequent treatment activities on sand beach communities were assessed (Deepwater Horizon Natural Resource Damage Assessment Trustees 2016). Many of the papers in this Theme Section describe the field studies conducted for injury assessment of coastal resources. However, no field studies were conducted for sand beach faunal communities impacted by the spill for several reasons. First, the

approach often used in past NRDA cases where sand beaches were oiled was to rely on: (1) maps of the degree of oiling based on data collected as part of the response to document exposure; (2) the literature to determine impacts by oiling category; and (3) the life histories of sand beach communities to predict recovery once the oil was removed. This approach, while expedient and effective for many spills, potentially underestimates the effects of extensive or intrusive response activities to the sand beach community. Second, the Trustees also had not anticipated the extended duration of repeated oil deposition nor the intensity and duration of response activities to remove the oil. This increased the complexity of integrating any potential sand beach faunal field studies over much of the northern Gulf of Mexico during the most critical time periods of the response. Finally, field survey study design was further complicated by a lack of baseline sand beach faunal community information for many of the impacted beaches. The Trustees determined it was more effective to consider alternative assessment strategies that relied on previously used techniques but also allowed them to consider intensity of disturbance on the beach community. Therefore, in the absence of any field measurements on species composition, abundance, or density in impacted versus control areas, the injury assessment had to be based on existing data, which consisted of (1) extensive documentation of oiling degree and duration, (2) detailed information on treatment methods, location, and duration, and (3)

estimated impacts to beach macrofaunal communities from a synthesis and review of the literature for similar types of oiling and disturbances.

Bejarano & Michel (2016) reviewed the oil spill literature on sand beaches and found that the extent of impacts and intervals to recovery vary according to the degree of oiling. Recovery intervals ranged from several weeks to several years, with longer recoveries for spills with long-term oil persistence or when there was no oil cleanup. Studies of 2 spills were considered most relevant because the oil weathered at sea during long-distance transport before stranding on shore: the 1979 *Ixtoc 1* blowout of crude oil in the Gulf of Mexico which affected similar fauna in southern Texas; and the 2002 TV 'Prestige' spill of heavy fuel oil off Spain. For the *Ixtoc 1* spill, Kindinger (1981) and Tunnell et al. (1982) report a wide range of survival of infauna from before and after the spill across the 13 beaches they sampled. At 7 of these 13 beaches, intertidal infauna abundances ranged from 85 to 97% lower after the spill. Three beaches showed a range from no change to modest increases in abundance (0–19%). The remaining beaches decreased in intertidal infaunal abundance (21–74%). For the 2002 TV 'Prestige' spill, de la Huz et al. (2005) and Junoy et al. (2005) reported decreases of 60 to 85% in the more abundant species; however, Junoy

et al. (2013, 2014) concluded that recovery occurred within 1.5 yr. Even these most relevant case studies have limited value to injury quantification because of the following unique conditions for the DWH spill: (1) the extensive spatial scale of affected beaches; (2) the contiguity of oiled beaches over long distances; and (3) the extended time interval over which beaches continued to receive oil. Furthermore, because the DWH spill response included extensive and intensive sand beach treatment for up to 4 yr after the initial oiling, we could not rely on the oil-impact literature alone for injury assessment. Therefore, we developed the concept of Response Injury (RI) as a category of sand beach injury to account for impacts from intensive and prolonged shoreline treatment to meet stringent shoreline cleanup endpoints during the DWH response, which had not been specifically addressed in previous injury assessments.

## METHODS

### Compilation of the types of response disturbance

Determination of response injuries followed a logical process (Fig. 2). The first step was to compile all available information on the types of response activi-

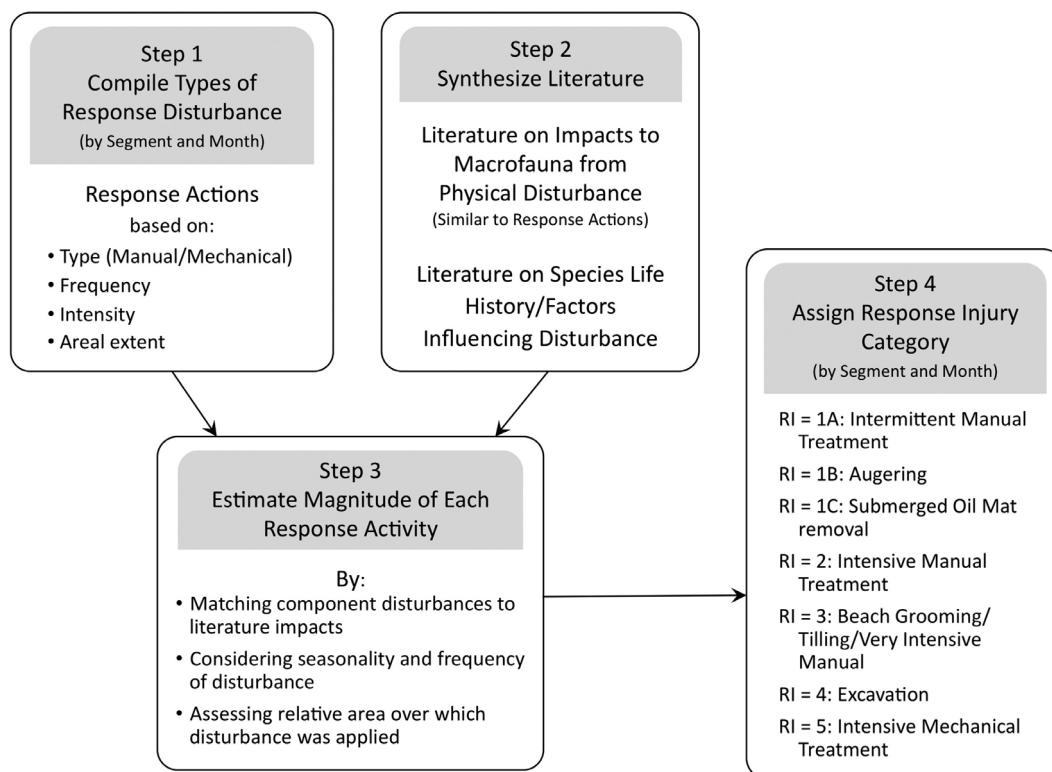


Fig. 2. Logical process for assigning Response Injury (RI) categories, as described in the 'Methods' (also see Table 3)



ties conducted on sand beaches that would disturb beach fauna. The Operations Branch of the Response Organisation generated daily to weekly reports that documented the number of workers, type of equipment, pounds of material removed, and other information for each shoreline segment by day. These reports were less organized early in the response; however, there were many other types of reports generated by monitors that were used as well. Shoreline treatments were assigned and tracked based on shoreline segments that were established by the Deepwater Horizon Unified Command very early in the response. In Florida, Alabama, and Mississippi, shoreline segments were usually about 500 m long; in Louisiana, they could be several kilometers long. We compiled treatment method and the number of times it was conducted on a segment per month into spreadsheets for each state.

### Literature compilation

Next (as shown in Fig. 2), an extensive array of published and unpublished documents was consulted to estimate the injuries induced by the types of response activities employed (described in detail by Michel et al. 2015). Literature searches were conducted using PubMed, Web of Science, Google Scholar, and other databases available through End-Note® online search tools. Each citation was reviewed to select those most applicable. The End-Note® library for the references that we reviewed included over 200 papers (Bejarano et al. 2015); however, herein we cite the 39 most relevant references. We summarized key biological information of sand beach fauna in the northern Gulf of Mexico by beach zone, association with and use of wrack, role in trophic transfer, reproductive mode (benthic-pelagic or brooding), seasonality, and prey. The importance of life history in predicting expected recovery from oiling and response injuries resulting from the DWH oil spill is discussed by Michel et al. (2015).

### Literature on sand beach communities

Based on our review of literature sand beach communities, Hooper (1981), Kindinger (1981), Rakocinski et al. (1991, 1998a,b), Yáñez-Arancibia & Day (2004),

Cobb & Arnold (2008), and Irlandi & Arnold (2008) provided the most information relevant to northern Gulf of Mexico beaches. These studies show that sand beach macroinvertebrates live in a high-energy environment with frequent sand movement, and display a high degree of spatial and temporal variability that is controlled, in part, by wave energy, beach slope, grain size, salinity, and organic content. Globally, many studies (e.g. McLachlan & Jaramillo 1995, Defeo & McLachlan 2005, Dugan et al. 2011, Schlacher et al. 2008) have demonstrated that the structure and nature of the habitat, fauna, and services offered by the supratidal and intertidal (= supralittoral and littoral) differ substantially (Fig. 3). Considering these 2 beach zones separately is also required for our purposes, as most mechanical beach cleanup activities occurred in the supratidal zone, with the notable exceptions of where intertidal and subtidal oil mats were removed and dredging occurred, although manual removal occurred in all zones. Thus, separating the beach into supratidal and intertidal zones allowed us to assign a response injury to the appropriate spatial beach component.

The supratidal zone is the elevated portion of the beach where wrack accumulates because it is infrequently inundated by tides and wave run-up. Wrack, which generally consists of *Sargassum*, *Spartina* stems, and subtidal vascular grasses in the northern Gulf of Mexico, supports a community of invertebrates consisting of terrestrial, semi-terrestrial, and marine species. The terrestrial species (air-breathing species for which the majority of the life cycle occurs in terrestrial or freshwater habitats) include insects

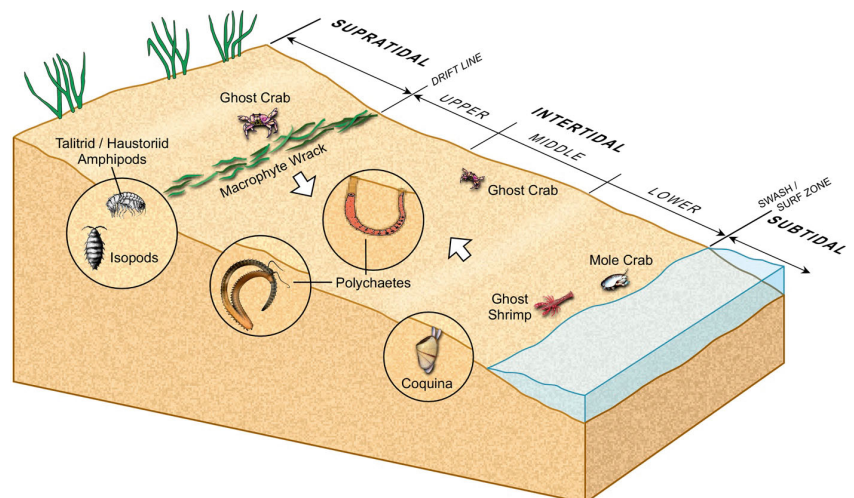


Fig. 3. Distribution of representative sand beach invertebrates. The 3 zones of the intertidal zone grade into each other and are not necessarily rigid in their extent. The fauna typical of the middle intertidal zone is a combination of those found in the upper and lower intertidal zones (represented by white arrows)

(springtails, flies, beetles, and ants) and chelicerates (spiders and mites). The semi-terrestrial species (air-breathing through moistened gills but dependent on saline waters for part of their life cycle) include several species of talitrid amphipods and ghost crabs. The marine species (water-breathing through gills and dependent on saline waters for all of their life cycle) include haustorid amphipods and some polychaetes. These organisms shred the wrack while feeding and are, themselves, often consumed by shorebirds, passerines, and mammals. Wrack-associated organisms may comprise up to 40% of intertidal species and represent an important prey source for higher trophic levels (Dugan et al. 2003). Shredding of the wrack produces fine particulate organic matter that subsequently is degraded by bacteria, releasing the bound nutrients. Predation of this community primarily directs carbon transfer into terrestrial food webs. The supratidal community includes ghost crabs as well, although they do not depend directly on wrack because they are omnivores capable of feeding on marine and terrestrial plants and animals as well as on carrion.

The intertidal beach community differs from the supratidal community in species composition, nutritional foundation, and fate of trophic transfer. The standing invertebrate biomass in the intertidal greatly exceeds that occurring in the supratidal zone

(Raffaelli et al. 1991, Colombini & Chelazzi 2003, Janssen & Mulder 2005). In the northern Gulf of Mexico, the intertidal benthic community consists entirely of marine species and is dominated by coquinas, mole crabs, polychaetes, and haustorid amphipods. The majority of these species are suspension feeders relying on beach or surf diatoms as their primary source of nutrition. They are important prey for shorebirds. Fish, especially juveniles of several species, prey on these invertebrates when the beach is inundated. Consequently, trophic transfer of carbon and nutrients from this part of the beach enters into both terrestrial and marine food webs.

### Literature on response activity impacts

For the different sand beach treatment activities used during the DWH response, component disturbances specific for that response were identified (Table 2), and the literature on similar types of disturbances was summarized. Intertidal communities on sand beaches are frequently considered tolerant to disturbances because the fauna are well-adapted to the dynamic beach environment. However, these fauna are directly impacted through cleanup operations by mortality from crushing and desiccation during sediment shifting and removal, and indirectly by

Table 2. Sand beach treatment method descriptions and expected associated impacts to sand beach biota and habitat. Intensity of disruption is represented as \* = minor; \*\* = moderate; \*\*\* = major

Description	Foot traffic	Vehicular traffic	Sifting mortality	Wrack removal	Biota removal	Biota desiccation	Wildlife disturbance	Substrate compaction	Shell removal	Equipment spills	Dune damage
<b>Manual treatment methods</b>											
Surface removal by shovels, rakes, sifting tools, and other hand tools to 15 cm	*	*	*	**	*	*	*	*	*		
Deep removal >15 cm deep	**	**		**	*	**	**	**			
Transport of workers, waste materials		*					*	*		*	*
<b>Mechanical treatment methods</b>											
Augering often in a 10 m grid spacing	**	**			*		**	*			
Beach cleaners/sifters to depths up to 50 cm	*	***	***	**	**		**	**	**	*	
Excavators to remove clean/oiled sand	*	***			**	**	**			*	
Stockpiling of clean sand overburden		**				**	**				
Heavy trucks for hauling sand to stationary sifters and back to excavation area		***					**	**		*	
Bulldozers to spread sand at excavation area		***					**	**		*	
Excavators to depths as great as 120 cm, often with wet sifting in screened bucket		***	***			**	**	**	**	*	
Tilling to 30 cm, often with multiple passes		**		**			**	*			*
Dredging							**				
Staging areas, where response equipment was transported on/off the shoreline	**	***					***	***		**	*

altering habitat suitability (substrate compaction, wrack and shell removal), disrupting reproduction and recruitment patterns, and removing food supplies. Response activities during the DWH response were characterized by the 4 categories described below, though we note that these categories will vary depending on the spill-specific response methods.

#### Foot traffic

Persistent human trampling on beaches results in reduced faunal abundances. Noriega et al. (2012) showed consistent 10-fold decreases in ghost crab abundances between visited and unvisited beaches (actual densities changed inversely with intensity and frequency of human activity). Moffett et al. (1998) demonstrated experimentally that barefoot human traffic reduced the survival of softer-bodied crustaceans and juvenile bivalves in the lower intertidal. Compared to other disturbances, foot traffic effects appear moderate (McLachlan & Brown 2006), although small losses in faunal abundances are consistently observed. Manual removal methods often involved intensive foot traffic (Fig. 4A) collateral with other disturbances (e.g. vehicular traffic, wrack removal, sifting) and occurred in all intertidal and supratidal zones.

#### Off-road vehicle traffic

Off-road traffic (4-wheelers, cars, and pickup trucks) on sand beaches has been studied extensively. These studies indicate that the effects of off-road traffic on local invertebrate assemblages vary greatly depending on their spatial and seasonal occurrence and abundance, and on their specific life histories. Schlacher et al. (2007) found that ghost crabs, with a soft exoskeleton, are frequently crushed by off-road traffic if their burrows are relatively shallow (5 cm); mortality declined exponentially with burrow depth. Those authors also found that ghost crab densities were higher in areas of low to moderate traffic, while individuals were smaller in heavily impacted areas, suggesting alterations of the population structure. Heavily trafficked beaches also had lower abundance, species richness, and diversity of intertidal macrobenthos, and strong changes in the community structure were driven by the low abundances of cirrolanid isopods (Schlacher et al. 2008). Direct crushing appeared to be the main cause of community change.



Fig. 4. Sand beach treatment methods. (A) Manual removal/sifting to 15 cm at Ft. Pickens, Florida, January 2011. (B) Four 'Sand Sharks' at Ft. Pickens State Park, running in tandem and sifting to 45 cm, February 2011. (C) Sifting of sediment piles created by mechanical removal on Grand Terre II, Louisiana, October 2010. (D) Excavation of oil mats at Elmers Island, Louisiana, December 2010



Lucrezi & Schlacher (2010) reported that sand beaches with traffic were slightly hotter and had lower moisture content than beaches closed to traffic, and not only were ghost crabs smaller, but also constructed much deeper and longer burrows, possibly to avoid desiccation. Kluft & Ginsberg (2009) demonstrated that vehicle traffic can degrade beach wrack quality by crushing, scattering, or burying, thus impacting the survival of invertebrates that depend on this habitat for food and shelter. Open-beach species (i.e. beach hopper and wolf spider) were more susceptible to disturbance than wrack inhabitants (enchytraeid oligochaetes and tethinid flies). Gastropods, on the other hand, appeared to be more resistant than soft-bodied invertebrates (mysids and isopods) to vehicle traffic (van der Merwe & van der Merwe 1991). Aside from direct crushing, heavy traffic decreases invertebrate abundance by reducing food availability (including wrack), increasing species displacement, disrupting the intertidal habitat and the physical properties of the sand substrate, and increasing invertebrate exposure to predators from the continuous maintenance of burrows (Schlacher et al. 2007, Kluft & Ginsberg 2009). Many of these factors in turn can influence recruitment.

Compaction increases the bulk density of the substrate and reduces the interstitial space, thereby altering the capillarity, water retention, permeability, and exchange of gases and nutrients within the substrate (USACE 1989, Defeo et al. 2009). Compaction also increases the resistance to burrowing, which can impact burrowing behavior and reduce the abundance of burrowing fauna (Lindquist & Manning 2001). The overall impacts of compaction can be translated into reduced substrate productivity and microhabitat suitability (Lindquist & Manning 2001).

#### Beach grooming and wrack removal

Some of the mechanical beach cleaners used during the DWH response (Fig. 4B) were modified from those used for beach grooming on amenity beaches. While operated in a similar manner for beach grooming, these machines were set to extend 30 to 45 cm into the beach sand rather than 0 to 15 cm used during normal grooming, thus increasing impacts to the infaunal community. Screens of 6 to 25 mm were used. Beach grooming has significant effects on the community structure (depressed species richness, abundance, and biomass) of wrack-associated fauna, causing substantial reduction of prey for higher trophic levels (Dugan et al. 2000, 2003, Defeo et al.

2009), and, depending on the spatial scale of grooming (<1 to 100 km), the effects can be noticed at scales ranging from weeks to years (Defeo et al. 2009). Mechanical raking (0–3 cm penetration) for wrack removal on the upper intertidal zone at Padre Island National Seashore, Texas, lowered the mean density and biomass of all macrofauna within 3 d of raking, and the density and biomass of the amphipod *Orchestia grillus* and polychaetes up to 10 d after raking, compared to unraked areas (Engelhard & Withers 1997).

Removal of wrack with mechanical beach cleaners at 2 tourist beaches reduced the percent total organic matter in the upper beach zone and caused high community stress (i.e. lowered invertebrate diversity, the number of distinctive taxa, and genetic diversity were caused by replacement of species with a higher number of opportunist species), compared to non-tourist beaches (Gheskiere et al. 2005). The removal of the top 5 cm of sand surface with mechanical beach cleaners (Gheskiere et al. 2006) caused significant changes in the total abundance and community structure immediately after cleaning by reducing the abundance of dominant nematode species and harpacticoid copepods, although they recovered completely in the following 2 tide cycles. In Sweden, cleaned beaches had a much lower level of organic carbon than un-cleaned beaches, and the most intensively cleaned beaches had lower total benthic biomass (Malm et al. 2004). However, biodiversity and community structure were not significantly different between cleaned and un-cleaned beaches.

Studies of the effects of beach cleaning in Poland suggested that trampling and mechanical cleaning may have contributed to the disappearance of air-breathing amphipods or sandhoppers from the most frequently visited beaches (Weslawski et al. 2000a,b and citations therein). Furthermore, wrack removal from the upper layer of sand and sand sifting through a 5 mm sieve effectively removed important food sources for key beach fauna, which are linked to the disappearance of macrofauna and the decline of their predators. Weslawski et al. (2000a) indicated foot traffic (3000 steps  $m^{-2} d^{-1}$ ) caused sufficient beach fragmentation and mixed debris with sediment down to 10 to 30 cm.

#### Sediment removal and placement

Cleanup operations involving translocation of large volumes of sand (Fig. 4C,D) can be equated with beach nourishment projects, as sand is

mechanically moved and redistributed on the beach surface, resulting in sizable changes in geomorphology (beach profile, sediment composition, substrate compaction), as well as in temporary changes to the beach inhabitants. Studies following beach nourishment projects and related activities found: (1) slow recovery of an intertidal clam (*Donax* spp.) population after a nourishment project that replaced the original substrate with sediment containing high levels of shell fragments (Peterson et al. 2000); (2) slow recoveries of macrobenthos after a nourishment project that increased concentration of fine sediments (Rakocinski et al. 1996); (3) large impacts on invertebrate populations for nourishment projects that coincide with the recruitment period of indicator species (Cobb & Arnold 2008); and (4) low species richness and equitability compared to pre-nourishment levels (Reilly & Bellis 1983). Major disruptions of the sand beach surface can have significant impacts at population (demography and dynamics), community (species richness), and ecosystem (functional processes, nutrient flux, trophic dynamics) levels (Defeo et al. 2009). Furthermore, reduction in the abundance and biomass of dominant species has been linked to disturbances in the foraging behavior of shorebirds and to reduced habitat productivity (Peterson et al. 2006, Defeo et al. 2009). However, others (Nelson & Collins 1987) have reported no measurable effects on indicator species attributable to nourishment projects.

Beach nourishment can cause immediate ecological damage to the resident sand beach invertebrate community including complete mortality of resident intertidal biota. Bilodeau & Bourgeois (2004) found that 2.5 yr after nourishment at the Isles Dernieres barrier islands of Louisiana, the ghost shrimp *Callinectes islagrande* did not have the large densities seen at reference sites with well-established populations. Only a few juveniles and 1 ovigerous female were found on the nourished beach, indicating that the population showed no indication of recolonization or recruitment. The lack of recolonization was attributed to changes in the sediment composition.

Mechanical disturbance, similar to that produced by many response activities, has been demonstrated to have direct, negative effects on beach macrofaunal populations. Lindquist & Manning (2001) evaluated impacts of beach nourishment and mechanical redistribution of beach sand (bulldozing) and found significant declines in the abundance of ghost crabs 6 to 8 mo post-bulldozing. Bulldozing also reduced the

abundance of mole crabs, though these changes were not statistically significant from controls. Other species (i.e. coquina clams, spionid polychaetes, and amphipods) appeared to have escaped the impacts of bulldozing as their abundances resembled those of control beaches. Peterson et al. (2000) found that both beach nourishment and bulldozing had quantifiable effects on intertidal species 5 to 10 wk post-treatment compared to control beaches. Nourishment reduced the density of 2 dominant taxa, mole crab and *Donax* spp. by 99 and 86%, respectively, possibly by altering the composition of the substrate, whereas bulldozing reduced the abundance of mole crabs and active ghost crab burrows by 37 and 65%, respectively, probably by changing beach morphology. Peterson et al. (2006) also attributed large mass mortality of benthic macroinfauna to beach filling. Over several months post-treatment, *Donax* spp. and amphipods had much higher abundances (85 and 89%, respectively), and ghost crab burrow density across the beach was up to twice as high on undisturbed control beaches. In contrast, ghost crab summertime recruitment appeared to have been inhibited on filled beaches. In Australia, beach nourishment caused the elimination of an amphipod with signs of recovery seen only 9 wk later (Jones et al. 2008). In South Africa, excavation of sand to a depth of 30 cm caused temporary changes in the abundance of macrofauna; this community required 7 to 16 d to recover following a single disturbance event (Schoeman et al. 2000).

From the literature, it is clear that species susceptibility to impacts from the types of disturbances similar to sand beach treatment methods is largely dependent on individual body size, fragility (soft vs. hard bodies), population turnover rates, and burrowing behavior (deep vs. shallow). Generally, large-scale operations would be more detrimental to species that: (1) brood their young; (2) have a soft exoskeleton; (3) have larger sizes and lower turnover rates; (4) build shallow burrows; (5) have seasonal reproductive cycles that coincide with cleaning activities; (6) occur at high densities in soft, non-compacted sand; and (7) are more closely associated with the substrate, and therefore are more strongly impacted by changes in the structure of the sand matrix (compaction). The fauna comprising sand beach communities along the northern Gulf of Mexico shoreline have many of these characteristics (Shelton & Robertson 1981, Britton & Morton 1989), and impacts would be proportional to both the temporal frequency and spatial extent of disturbances.



### RI assignment to the shoreline

We developed 5 RI categories for the DWH response (shown in Fig. 2 and defined in Table 3) that depended on disturbance type, intensity of the disturbance, the frequency of use, and, based on a literature review, the likely effects of each specific disturbance. The scale is ordinal; actual impacts vary substantially within each category. The compiled data on shoreline treatment methods by segment and date were used to assign a monthly RI category for each segment.

For each month and segment, we derived an estimate for the impacts to sand beach ecosystem services by considering: (1) the levels of intensity of the response activities; (2) where the activity occurred on the beach; (3) whether the area where the activity was applied was spatially extensive or narrowly

restricted, and (4) whether the activity was coincident with oiling of the beach (May to September 2010).

For both intermittent and intensive manual removal (RI = 1A and 2), during the period when the oil was coming ashore, cleanup crews aggressively removed all wrack, whether it was visibly oiled or not. Removal of wrack would have also removed many wrack fauna and their essential habitat. Consequently, in cases where complete or near complete removal of the wrack and associated fauna occurred, there would be a very high loss of the faunal community in the supratidal. Wrack removal or disturbance along 100s of km of contiguous shoreline would have far greater faunal impacts, and ecosystem recovery would be much slower, than those occurring along isolated beaches disturbed on smaller spatial scales (S. Fegley & J. Michel unpubl.). Later, when monitors

Table 3. Response Injury (RI 1–5) categories, descriptions, intensity by the 4 major disturbance types, and overall impacts on sand beach ecosystem services

RI	Description	Foot traffic	Vehicular traffic	Sediment disruption/ excavation	Wrack removal	Overall impacts to beach fauna
1A: intermittent manual treatment	Manual only, lower frequency (<20 visits mo <sup>-1</sup> ), includes vehicle traffic for transport of workers and waste	Very low to low	None to low, depending on how many vehicles were present	None to low	Prior to 1 October 2010 - high;	High
					After 1 October 2010 - low	Very low (non-amenity beaches only)
1B: augering	Mechanical augering	Low to moderate	High due to use of the tracked auger	Very high in augered pits	Low. Incidental physical disruption only	Very low
1C: submerged oil mat removal	Long-armed backhoes on tracks operating in the intertidal zone at the water line to remove subtidal oil mats	Moderate	High due to use of tracked vehicles and towed equipment	High	None to very low	Low
2: intensive manual treatment	Mostly manual (but includes walk-behind sifters for any duration), higher frequency (>20 visits mo <sup>-1</sup> ), includes vehicle traffic for transport of workers and waste	Moderate	None to low, depending on how many vehicles were present	None to low	Prior to 1 October 2010 – high	High
					After 1 October 2010 - low	Very low (non-amenity beaches only)
3: beach grooming/ tilling/very intensive manual	Treatment at least twice mo <sup>-1</sup> with a mechanical beach groomer that would sift the sand to a depth of 30 cm; all tilling operations; intensive manual removal of large amounts of oiled materials	Moderate	High	High	High due to direct removal and physical disruption	High
4: excavation	Treatment at least twice mo <sup>-1</sup> with a mechanical device, and at least once mo <sup>-1</sup> beach sand was mechanically removed from the beach and sifted; mechanical removal of clean sand for manual removal of oiled sand	Moderate	High	High	High	High
5: intensive mechanical treatment	Extensive deep (>30 cm) mechanical treatment, staging areas, and dredging	Moderate	High	High	High	High

were assigned to the cleanup crews, clean wrack removal was minimized, losses to the faunal community would be reduced, but only to non-amenity beaches, because amenity beaches had historically experienced persistent disturbances associated with foot traffic and beach cleaning. For submerged mat removal (RI = 1C), which involved tracked backhoes operating in the intertidal zone, losses would be low because the repeated movement of heavy equipment was limited to small areas of each segment.

More intensive treatments defined in Table 3 include beach grooming/tilling/very intensive manual treatment (RI = 3), excavation (RI = 4), and intensive mechanical treatment/staging areas/dredging (RI = 5) and would have induced extensive mortality within the treatment area by displacement, physical trauma, desiccation, and burial during sand storage and replacement. These activities also included ancillary, extensive foot/vehicle traffic and wrack removal. The extensive use of heavy equipment, such as large dump trucks, tracked backhoes, towed sifters, and bulldozers, during the DWH response was unprecedented in terms of both the spatial extent and intensity (Fig. 4B–D).

## RESULTS

Of the 965 km of sand beaches that were oiled, shoreline treatment was documented on 683 km (71%). In Florida, where nearly 80% of the oiling on sand beaches was classified as light and 43% as light persistent, frequent manual treatment (RI = 2) was conducted on 67% of the shoreline (Fig. 5). However, to meet stringent cleanup endpoints for amenity beaches, beach grooming and tilling were conducted in the supratidal along nearly 60 km that had heavier oiling initially but was subsequently buried. In Alabama, there were 2 areas where intensive treatment in the supratidal zone was conducted to remove deeply buried oil that caused persistent re-oiling of the beach. In Mississippi, much of the heavier oiling occurred on the outer barrier islands of the Gulf Island National Seashore, where mechanical methods were restricted. Thus, there was proportionally less response-related impact. On the sand beaches of Louisiana, in spite of over 80 km of heavier persistent oiling, intensive mechanical treatment was conducted on only 32.4 km because of concerns that mechanical treatment would increase

erosion on highly erosional islands. Furthermore, because of the remoteness and difficulty of access to many of the barrier islands in Louisiana, only manual removal (RI = 1 and 2) occurred on 140 km, regardless of the degree of oiling. It was only in 2011 and 2012, when the decision was made to conduct mechanical and intensive manual removal to prevent chronic re-oiling, that RI = 5 categories were assigned (Fig. 6).

The maximum RI category for each shoreline segment varied for each year from 2010 to 2014, as did the contiguous extent of treatment in each year (Fig. 6). In 2010, treatments with  $RI \geq 2$  were conducted throughout the region, with mechanical excavation conducted late in the year along beaches in Florida and Louisiana. In 2011, persistent buried oil was removed by excavation and sifting along Florida and Alabama beaches, and deep excavations were conducted at Fourchon, Louisiana (RI = 5, red lines in

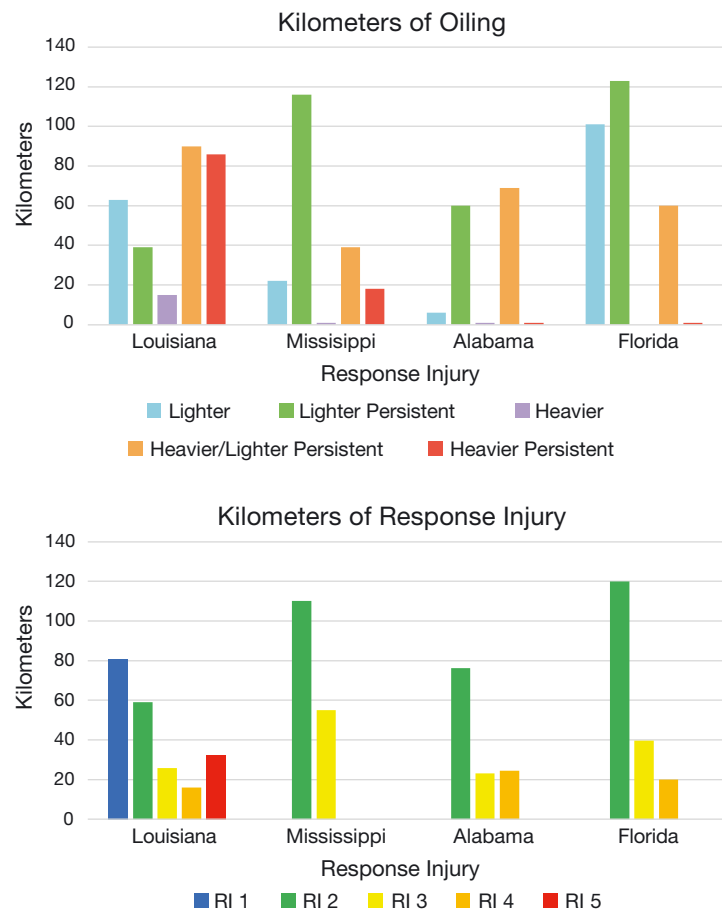


Fig. 5. Length of sand beach by state-assigned (A) oiling category and (B) maximum Response Injury (RI) category. 'Persistent' applies to oiling that persisted on beach for at least 26 wk based on repeat surveys. See Nixon et al. (2016) for more detail on how oiling categories were defined and determined

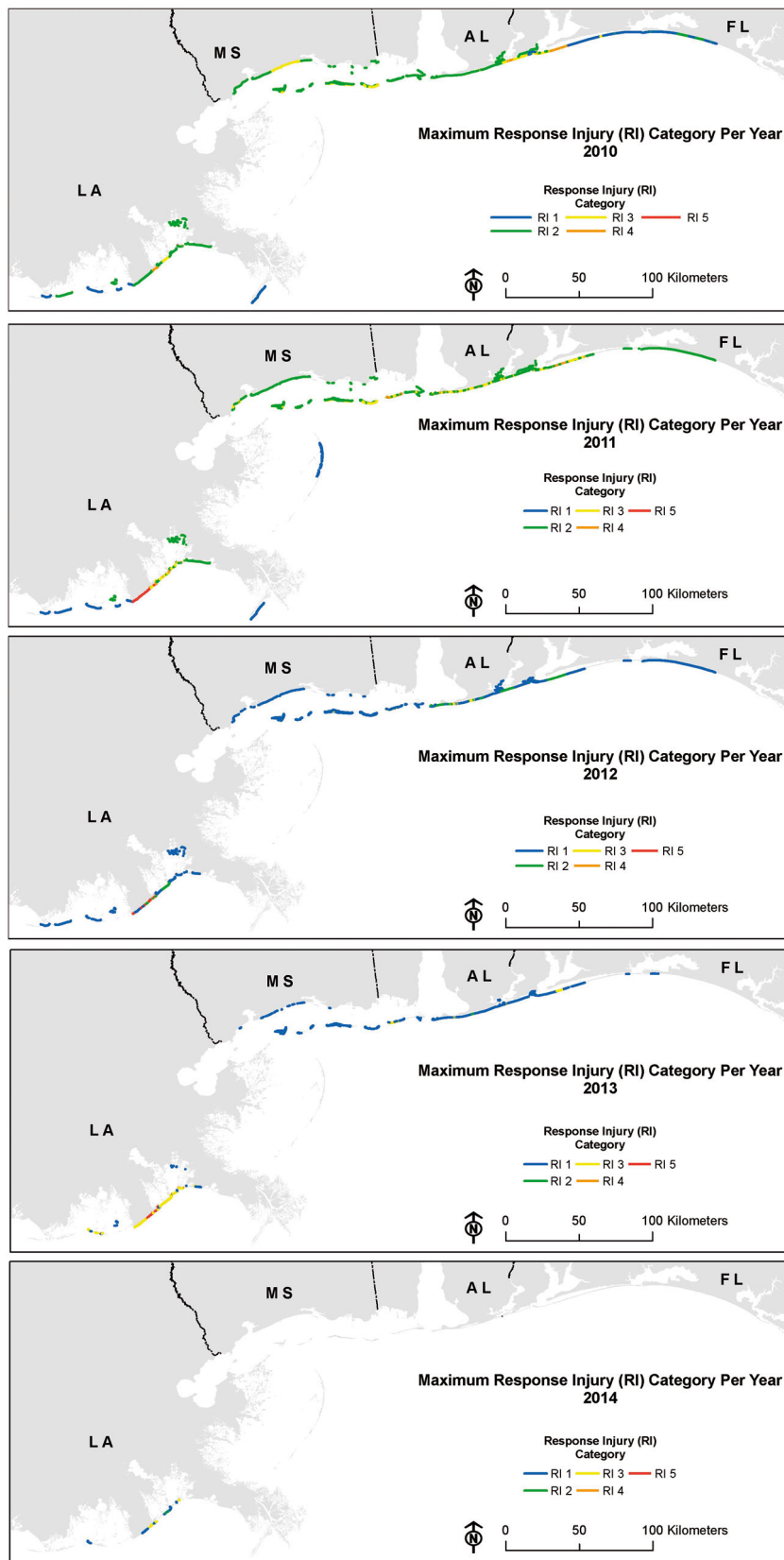
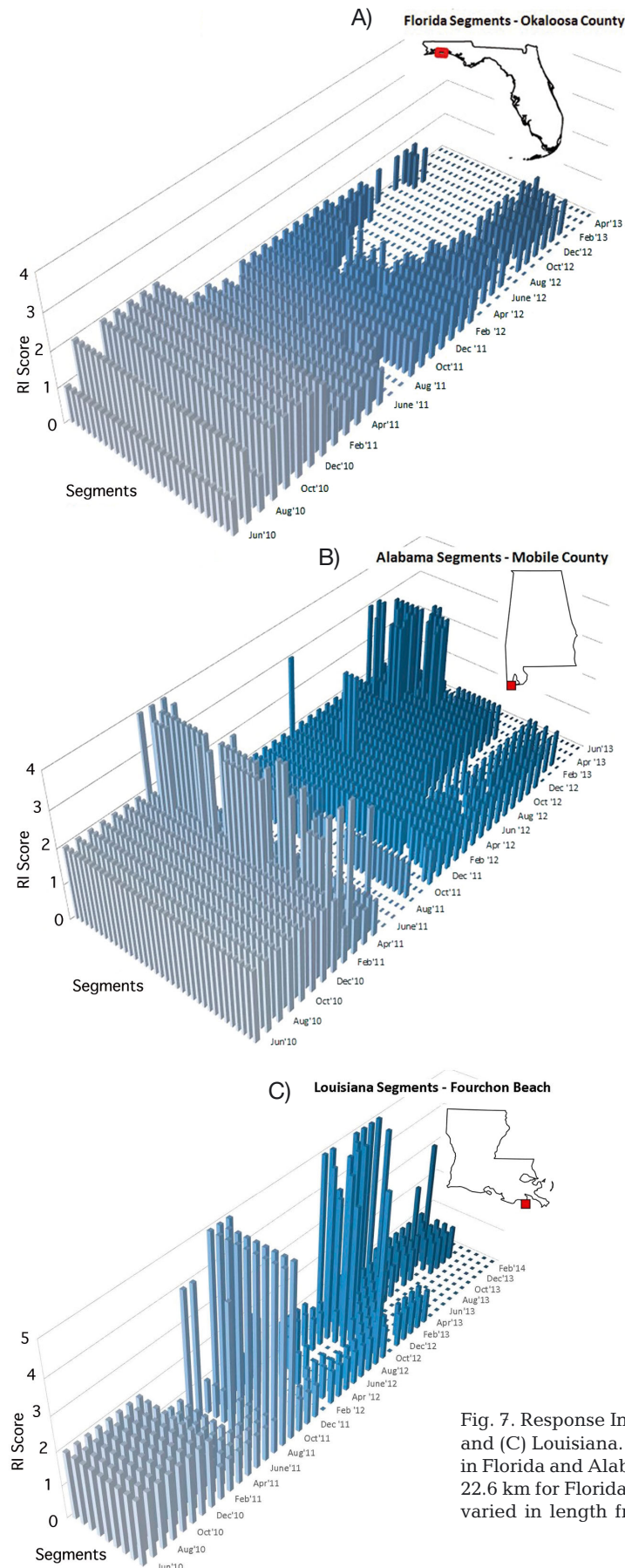


Fig. 6. Maximum Response Injury (RI) category for each beach segment per year

Fig. 6). By 2012, mostly manual removal methods were being conducted, except for some locations in Louisiana. By 2013, intermittent manual removal methods were conducted in Florida to Mississippi, ending in June 2013 when treatment operations were officially terminated on all of these beaches. However, mechanical excavation and intensive manual removal methods were being conducted later in 2013 along Louisiana beaches with persistent buried oil. Louisiana beaches were moved out of response by April 2014, after intensive manual removal of oil on some beaches.

The range of intensity and duration of treatments, and consequently RI and timing for recovery, varied over time and space, which can be illustrated by examining 3 representative areas (Fig. 7). For the lightly oiled beaches of Florida at the eastern extent of shoreline oiling (Fig. 7A), only manual methods were used in all tidal zones, initially at frequencies  $>20$  visits  $\text{mo}^{-1}$  (RI = 2) until January 2011, when these segments moved into a 'patrol and maintenance' period of less frequent surveys. Workers conducted cleanup operations on every beach segment for 12 mo and on most segments for 19 mo.

Along Alabama beaches, treatment patterns differed. Frequent manual removal only was allowed until early 2011, when there was a push to remove the buried oil. Deep excavations and sifting occurred in the supratidal zone on Dauphin Island, which included large staging and sifting operations, and West Point Island (plotted on the left side of the chart) that lasted from January to March 2011 (Fig. 7B). These types of operations are shown in Fig. 4C. Note the absence of any RI in summer of 2011; these segments were put on 'environmental hold' during bird nesting periods. However, chronic re-oiling on West Point



Island triggered the decision to conduct a second period of intensive mechanical treatment starting in December 2012. Therefore, West Point Island received multiple intensive mechanical removal operations, which would re-set any ecosystem recovery that had occurred after the initial oiling or earlier response activities.

Fourchon Beach, Louisiana, received the heaviest oiling and most intensive treatment (Figs. 6 & 7C). The pattern of treatment shows frequent manual removal (which included extensive vehicular traffic on this beach due to limited access) for >1 yr. These efforts were followed by intensive removal of buried oil in all tidal zones that was partially exposed during Tropical Storm 'Lee' in September 2011 and Hurricane 'Isaac' in August 2012.

These 3 examples (Fig. 7) show the varieties in type, spatial continuity, and duration of sand beach treatments following the DWH oil spill that were conducted for a period of 3 to 4 yr after the oil initially stranded onshore. These operations extended the impacts of the response well beyond that expected from oiling alone. Many studies have found that the sand beach invertebrate community recovers within 0.5 to 5 yr post-spill (as summarized in Bejarano et al. 2011 and references therein). Such recoveries, however, would be interrupted by additional impacts resulting from response activities. The RI impacts to the invertebrate community can exceed those associated with oil exposure (Whitfield 2003, de la Huz et al. 2005, Borzone & Rosa 2009), particularly where intrusive methods were conducted on beaches that were only lightly oiled. The recovery of sand beach invertebrate communities from the DWH spill event was predicted to have been delayed by 2 to 6 yr after the last response action (Deepwater Horizon Natural Resource Damage Assessment Trustees 2016).

## DISCUSSION

This work describes a semi-quantitative approach to incorporate impacts to sand beach habitats resulting from shoreline treatment activities, particularly where such activities are very intensive and frequent for long periods of time and along exten-

Fig. 7. Response Injury (RI) categories for segments in (A) Florida, (B) Alabama, and (C) Louisiana. Shading is used to improve visualization only. Each segment in Florida and Alabama is about 0.5 km in length, and the entire length shown is 22.6 km for Florida and 18.4 km for Alabama. The segments on Fourchon Beach varied in length from 1.7 to 5.5 km, and the entire length shown is 24.5 km



sive, contiguous lengths of shoreline. The DWH spill was the first case that we are aware of where response injury was determined in addition to and separate from injury associated with the oil exposure for sand beaches. This approach was needed because of the rapid and deep burial of the oil, which resulted in very intensive disturbances to the supratidal zone during subsequent buried oil removal. Most oil spills do not directly affect the supratidal zone (with notable exceptions); therefore, the supratidal sand beach communities are often not included in impact assessments. Only 1 study specifically mentioned the intensity of cleaning activity (heavy machinery and sediment and wrack removal during the 2002 TV 'Prestige' spill in Spain) in addition to the degree of oiling of the beaches that were studied. Macrofauna of the supratidal zone in heavily oiled areas were even more affected where grooming and wrack removal was intense (de la Huz et al. 2005). With the lack of studies on the impacts of intensive and extensive treatment of oiled beaches, our approach of using response data and the disturbance literature provides the only alternative.

We assigned impacts to beach fauna for the different RI categories in relative terms from very low to very high, and we specifically differentiated between amenity beaches (which have heavy foot traffic from high public use and regular beach manicuring that would remove wrack and shallow fauna) and non-amenity beaches (Table 3). For injury quantification for a specific spill, these impacts would be translated into a percent reduction of ecosystem services, followed by appropriate recovery rates depending on the life histories of the beach macrofauna. For each case, it will be important to consider the timing and areal extent of disturbances during intensive beach treatments in determining the recovery rates. Recovery from disturbances with small footprints can be very quick, with recruitment from adjacent, undisturbed areas, as demonstrated in the small, experimental study of the impacts of a single wrack removal event by Engelhard & Withers (1997), who found no significant differences in macrofauna density between raked and unraked areas 2 wk after disturbance ended.

During the DWH response, treatments were conducted along extensive lengths of shoreline and throughout the year. The types of equipment used were of similar or greater weight and footprint, and were involved in activities of greater intensity (both spatially and temporally), than those identified in the literature that we evaluated. As the frequency, intensity, and spatial extent of beach disturbance docu-

mented in the aftermath of the DWH oiling exceed the disturbances reported by the majority of studies of off-road vehicles, small field experiments, beach grooming, or one-time beach nourishment projects, greater impacts to sand beach communities can be expected to have occurred on Gulf of Mexico beaches receiving treatment. In consideration of all these factors, we argue that our rankings are conservative and may not fully account for even more extensive impacts originating from beach response treatment activities.

The deliberate, intensive treatment of sand beaches, particularly on amenity beaches, reflects the higher valuation of recreation over other ecological services in high public use areas. Where ecological services are deemed as (or more) valuable than recreation, such as on national parks and wildlife refuges, clean-up methods during the DWH response were much less intensive. The perception appears to be that sand beach communities are tolerant to disturbances and have relatively quick and predictable recovery rates. However, Bejarano & Michel (2016) found few studies of the short-term effects of oil on sand beach invertebrates, and even fewer that documented recovery. Oil exposure and physical disturbances to 965 and 683 km, respectively, of nearly contiguous beach shoreline in the northern Gulf of Mexico likely far exceed the damages documented in individual studies of small areas over short time periods.

The ecological consequences of intensive treatment of sand beaches as we have hypothesized definitely need to be confirmed through well-designed field studies. Results from such studies can and will be used during future responses, to make informed decisions about the tradeoffs in selecting the most appropriate response options. One of the best examples is the study of the severe impacts from use of high-pressure, hot-water flushing on rocky intertidal habitats during the TV 'Exxon Valdez' oil spill versus oil alone, made possible through designation of 'set-aside' study sites where no flushing was conducted (Peterson 2001, Shigenaka 2014). Since then, high-pressure and high-temperature flushing methods are mostly used only on man-made structures such as seawalls and riprap. Studies of various treatment methods on marshes have well documented the impacts of treatment, with the result that often natural attenuation is the preferred response option (Michel & Rutherford 2014). Without studies documenting the impacts of aggressive mechanical removal of oil from sand beaches, it will be difficult to argue for leaving some oil for natural attenuation, particularly on beaches with high ecological value.



Unfortunately, on high-use amenity beaches, the tradeoff analysis is based on economic impacts rather than ecological impact and recovery.

When shoreline treatment is likely to include intensive methods to meet cleanup endpoints, it is important to document the activity as soon as possible. For the DWH response, detailed documentation only occurred during the second year of the response, challenging reconstruction of segment-specific treatment history. Just as teams conduct surveys to document oiling conditions, initially and over time, it is equally important to document the temporal and spatial extent of intensive response actions.

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# Integrated effects of the *Deepwater Horizon* oil spill on nearshore ecosystems

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**ABSTRACT:** The interconnected nearshore habitats of the northern Gulf of Mexico provide refuge and feeding opportunities for fish and wildlife, including open water, shoreline, and terrestrial species. The *Deepwater Horizon* oil spill natural resource damage assessment documented injury as a result of the oiling of over 2113 km (1300 miles) of shoreline over an 87 d release from the wellhead. Field and laboratory studies indicate that oil degraded the health of coastal marsh vegetation and associated fauna, resulted in the loss of nearshore oyster cover, and increased erosion of oiled marsh edge habitat over approximately 174 km (108 miles) of shoreline. Sand beach habitat, submersed aquatic vegetation, and subtidal oysters were injured by a combination of oiling and response actions. The loss of billions of oysters resulted in failed recruitment over several years in the most severely affected areas (Barataria Bay, Black Bay/Breton Sound, and Mississippi Sound). Affected ecosystem services include supporting services (e.g. primary production) and provisioning services (e.g. fish and invertebrate abundance). Loss of vegetation and nearshore oysters and increased shoreline erosion may have disrupted regulating services associated with stable marsh (e.g. coastal storm and flood protection). The loss of marsh vegetation and oysters likely reduced nutrient cycling and water filtration services. Recovery of natural resources may take more than 20 yr in some areas. To prepare for future spills, we recommend that natural resource trustees develop generic conceptual models and prepare integrated injury assessment approaches for nearshore habitats to facilitate future injury quantification. Additional exploration of the trade-offs between response options could minimize or shift natural resource injury for future spills.

**KEY WORDS:** Mobile drilling unit · Natural resource damage assessment · NRDA · Salt marsh · Nearshore · Oyster · Aquatic vegetation

## INTRODUCTION

The 20 April 2010 explosion, subsequent fire, and sinking of the *Deepwater Horizon* (DWH) mobile drilling unit triggered a massive release of oil from BP's Macondo well. Initial efforts to cap the well were unsuccessful, and the well continuously released oil into the northern Gulf of Mexico for 87 d after the explosion. Oil from this release first approached nearshore areas in May 2010.

The Gulf Coast contains some of the world's most biologically diverse and interconnected habitats, in-

cluding coastal salt marshes, mangroves, estuaries, sand beaches, dunes, submersed aquatic vegetation (SAV), and barrier islands. These nearshore habitats form a complex mosaic of structural refuge and foraging habitat for fish, invertebrates, terrestrial animals, and migratory birds. The assessment of injuries from the DWH spill focused on the health of these habitats to meet the requirements of the Oil Pollution Act of 1990 (OPA) regulations on natural resource damage assessment (NRDA). Indicators of injury were selected for each nearshore habitat type, with greater emphasis on habitats that received the great-

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<sup>§</sup>Advance View was available online December 14, 2016

est extent and magnitude of oiling (e.g. marsh surface and edge communities).

NRDA is a legal process to determine injuries to public natural resources following oil spills or the release of hazardous substances, and to plan and implement an approach for restoring those resources. The goal of an NRDA is to assess the severity and duration of injury to natural resources and determine appropriate restoration of the injured environment. Trustee entities (federal, state, or tribal) must document connections between the release of, pathways for, and exposure to oil or hazardous substances (and response actions taken to respond to the spill); and the extent, magnitude, and duration of injuries. Injury quantification entails determining the degree, and spatial and temporal extent, of injuries relative to the condition the natural resources would have been in had the oil spill not occurred (i.e. baseline). Oil can injure natural resources through toxicity and physical smothering. Response actions such as removing contaminated sand or oiled wrack, flushing or raking marsh shorelines, or releasing river water in an attempt to keep oil from stranding can also injure habitats and natural resources, and responsible parties are liable for these injuries. Restoration is intended to compensate the public for lost services provided by natural resources and return the injured resources to baseline conditions. To determine baseline conditions, trustees use information on conditions before the spill, trends over time, and/or data from appropriate reference sites.

Natural resource trustee agencies for the DWH NRDA include the US Department of Commerce (NOAA), US Department of the Interior, US Environmental Protection Agency, US Department of Agriculture, and the states of Alabama, Florida, Louisiana, Mississippi, and Texas. Trustee agencies vary according to the natural resources affected by a particular spill. Trustee responsibilities include assessing injuries to natural resources, negotiating settlements with responsible parties, and implementing or overseeing restoration actions to compensate for injury.

The objectives of this synthesis are to integrate the findings of the DWH NRDA injury studies for nearshore habitats and to present considerations for future oil spill injury assessments. First, the assessment approach for the DWH NRDA will be discussed, followed by a summary of injury findings. These efforts have informed considerations for future spills, which will be presented last. In order to assess community- and ecosystem-level injuries, future assessments should evaluate multiple injury indicators at

the same locations and extend them to other areas based on exposure conditions (e.g. polycyclic aromatic hydrocarbon [PAH] concentrations and shoreline oiling extent and magnitude).

## ASSESSMENT APPROACH

For the DWH NRDA, the trustees developed a series of interconnected conceptual models for coastal wetlands, oyster reef, beach, and SAV nearshore habitats affected by the spill and response actions (see Figs. 1 & 2 as examples). Because of the large geographic and temporal scale of the spill and the numerous habitats and species potentially injured, the environment was divided into habitat and species groups to plan and implement assessment activities. Findings from nearshore habitats were then interpreted together to consider the integrated effects of shoreline and sediment oiling, while nearshore water column injury was integrated with the assessment of offshore water column injury. Injuries to birds, marine mammals, and sea turtles were evaluated through independent assessment activities. Findings from water column, nearshore bird, coastal dolphin, and nesting sea turtle studies will be briefly described here as additional indicators of ecosystem-scale injury.

In developing a pathway model to support study designs (Fig. 2), the trustees applied criteria based on requirements of the OPA NRDA regulations to identify representative species that may have been exposed and injured. Criteria for selecting species included representation of multiple trophic levels and the potential ability to detect adverse change from baseline conditions, to quantify and scale injuries, and to identify restoration projects that could compensate for injuries. Based on experience with other NRDA, injuries indicated by measurements with high natural variability (e.g. community metrics and many indicators of microbial processes) are difficult to relate to contaminant exposures, and were therefore excluded from consideration. Although there is increasing interest in evaluating ecosystem-level injury from human activities (MEA 2005), many ecosystem services are difficult to directly measure and quantify. Therefore, individual indicators of injury to ecosystem services were used to create a 'weight of evidence' for ecosystem-level injury.

The conceptual models were used to develop testable hypotheses to evaluate the relationship between exposure to oil or response actions and



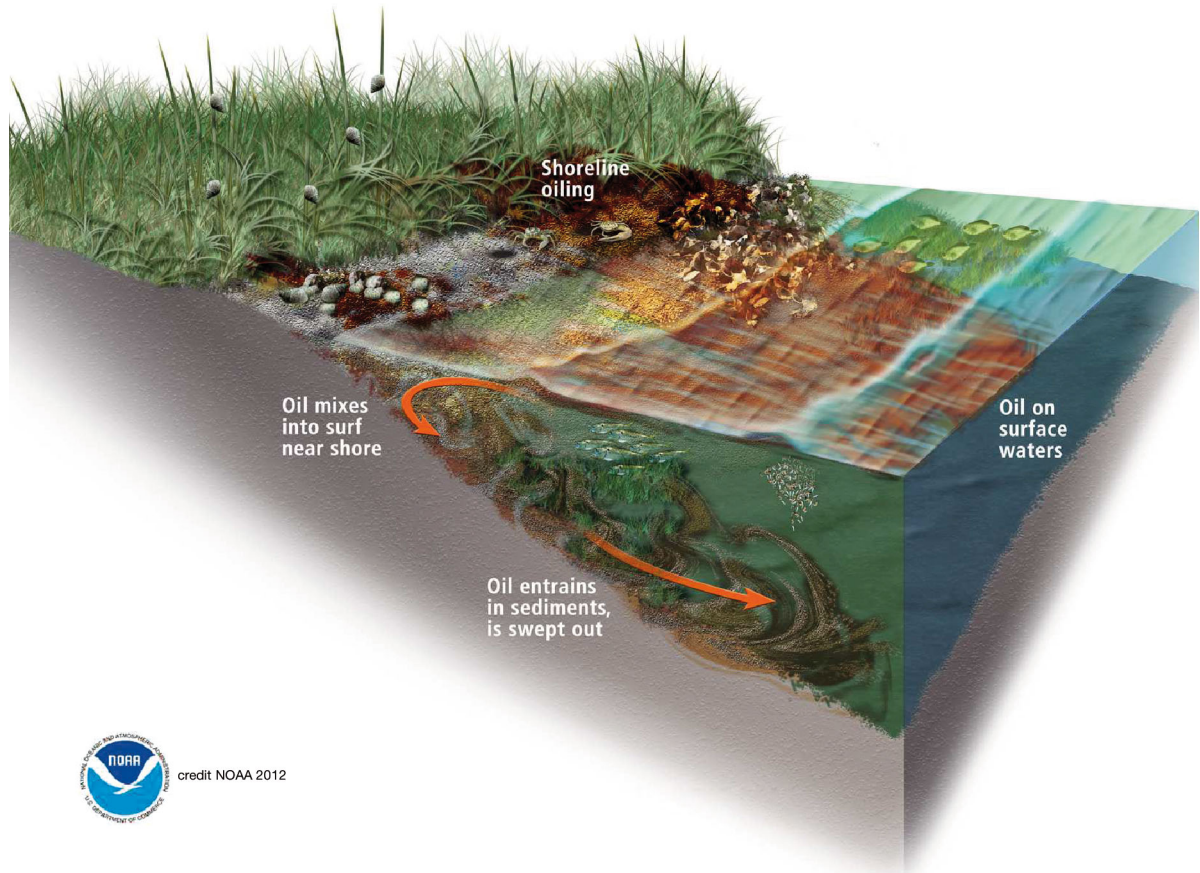


Fig. 1. Pathway for oil to reach nearshore habitats

injury metrics. For example, plant stem oiling was evaluated as the main possible driver of injury to marsh vegetation (Hester et al. 2016). Degree of shoreline oiling and associated response actions were hypothesized to be related to injury to near-shore and shoreline fauna and beach and SAV habitats (Zengel et al. 2016a, Powers & Scyphers 2016; Kenworthy et al. 2017, Michel et al. 2017, Powers et al. 2017, all this Theme Section). Subtidal oysters were hypothesized to be affected by a combination of oiling of surface water or shoreline and exposure to river water released in an attempt to keep oil from reaching the shoreline (Powers et al. 2015).

To test the hypotheses that resulted from the development of the conceptual models, the trustees compiled evidence of exposure to oil or response actions that could result in injury to habitats and species. Exposure to oil stranded on the shore was the primary factor resulting in injury to beach and marsh habitats and associated fauna. Shoreline oiling was evaluated using a combination of linear shoreline surveys (Shoreline Cleanup Assessment Technique) conducted as part of response actions and similar linear shoreline surveys conducted by

trustees in the summer of 2010. In addition, a 'pre-assessment' survey was conducted through the summer of 2010 to document shoreline oiling exposure and inform field sample design to quantify injury to marsh habitat (NOAA 2010). In this survey, plant stem oiling observations were gathered at targeted point locations to represent each shoreline oiling class as determined for this NRDA (see Nixon et al. 2016 for a description of oil classes). Aggregated oil on surface water was also considered as an exposure variable in evaluating injury to SAV and subtidal oyster habitats. Satellite- and aircraft-based remote sensing and aerial imagery were used to determine the number of days when surface water oiling was present in proximity to SAV and subtidal oysters (NOAA 2011).

In addition to the toxic, smothering, or fouling effects of oil, oil spill response actions also had the potential to injure nearshore habitats and species. The effects of physical disturbance such as trampling of vegetated shorelines, removal of sand and wrack from beaches, stranding of boom on shoreline habitats, building of sand berms, and the release of freshwater were evaluated. Exposure to response actions



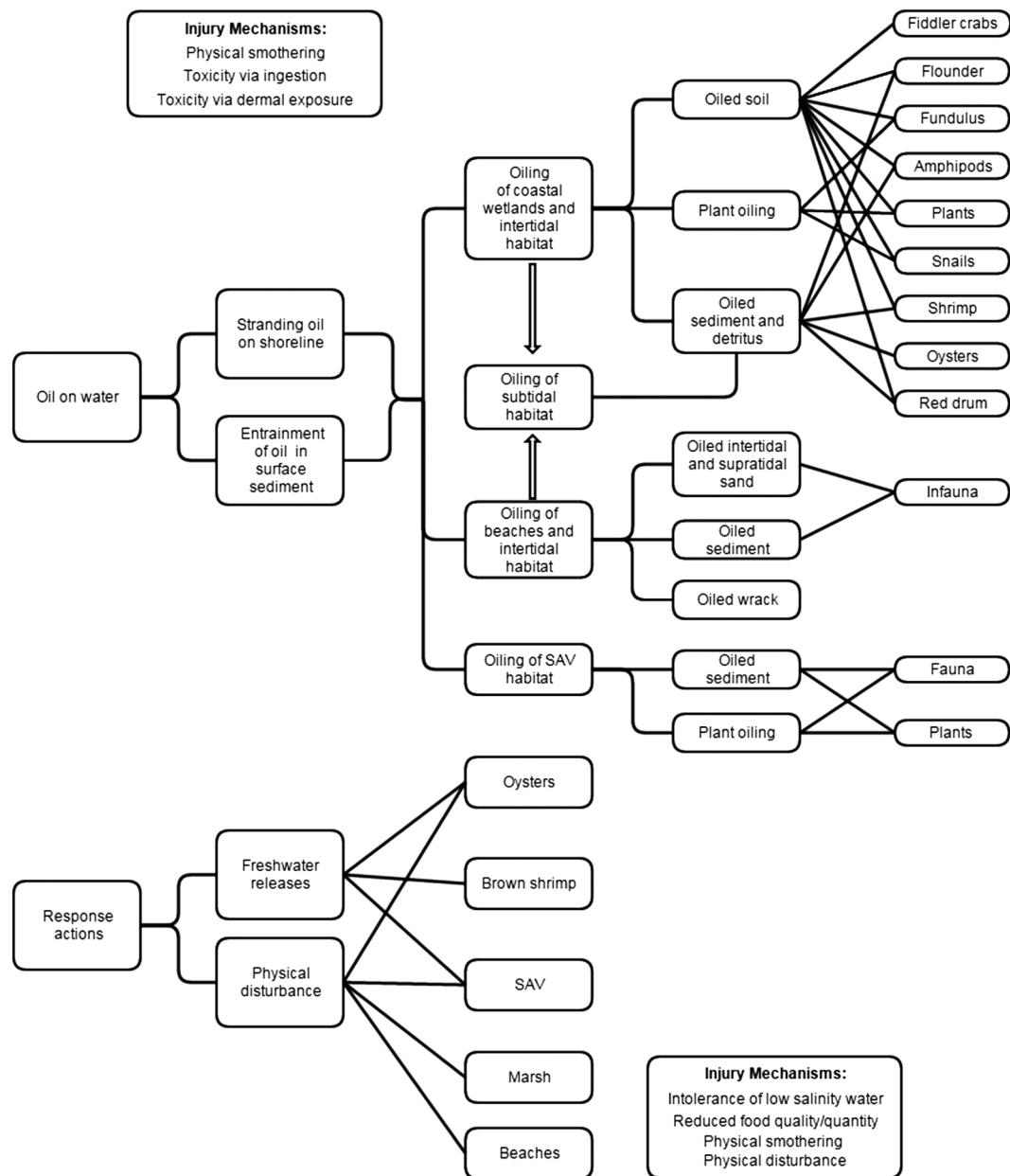


Fig. 2. Pathways of exposure for representative species to oil and response actions, and mechanisms of injury. Most resources were exposed via multiple pathways. Effects of nearshore water column exposure were considered in a separate assessment. ‘Sediment’ refers to submerged (intertidal or subtidal) substrates. Wetland ‘soil’ refers to upland or intertidal substrate colonized by vegetation. SAV: submerged aquatic vegetation

was evaluated using information on locations of boom that became stranded in marsh habitats, records of shoreline treatment on beaches and in marshes (type of treatment, timing, and location), and salinity observations in areas potentially affected by diversion of river water. Salinity observations from a variety of sources were compiled and interpolated to assist in interpreting effects on marsh fauna and nearshore and subtidal oyster abundance and

recruitment (McDonald et al. 2015, Powers et al. 2015).

Injury to coastal wetland habitats was assessed by evaluating the health and productivity of vegetation and fauna, as well as erosion of the marsh edge. Coastal wetland sampling locations were selected using a stratified random design representing a range of plant stem oiling conditions (Hester et al. 2016). The design included 4 coastal wetland habitat

types: mainland herbaceous salt marsh, back barrier salt marsh, mangrove, and the *Phragmites australis*-dominated marshes of the Mississippi River Deltaic Plain. Injury to marsh fauna was evaluated using shoreline oiling and/or soil PAH chemistry collected at vegetation sampling sites as exposure variables (Powers & Scyphers 2016, Zengel et al. 2016a,b, Powers et al. 2017). Individual species and life stages were selected from different trophic levels as indicators of a healthy functioning nearshore Gulf ecosystem. Known sensitivity to oil and proximity to oiled environments based on life-history information was also considered in selecting species and life stages for assessment. Marsh fauna included crabs, snails, shrimp, resident fish, burrowing infauna, and nearshore oysters that form emergent reefs or smaller hummocks that fringe marsh edges. Growth, survival, and reproduction were evaluated using a combination of field and laboratory techniques. Periwinkle snails *Littoraria irrorata* were studied in heavily oiled areas and sites representing reference conditions (Zengel et al. 2016a). Nearshore eastern oysters *Crassostrea virginica* were collected from sites representing a range of shoreline oiling and salinity conditions (Powers et al. 2017). Records on locations, methods, severity, and duration of cleanup actions compiled by the US Coast Guard and other response agencies were used to classify sampling sites as to whether they were likely to have been disturbed by response actions. Where feasible, sampling stations for different metrics were co-located so injuries could be compared and related to each other (e.g. vegetation health, shoreline erosion, and nearshore oyster cover and abundance).

Because trustees must distinguish effects associated with the incident from those caused by other environmental factors, reference site locations were randomly selected from the same habitat type and hydrologic regime as oiled sites. Coastal wetland sampling locations were selected in the late summer/early fall of 2010 and formed the basis for sampling periwinkles, fiddler crabs (*Uca* spp.), and nearshore oysters. Data were collected to evaluate possible confounding factors that could influence the injury metrics relating to vegetation and faunal health and marsh structure. For example, coastal Louisiana marshes have a high rate of baseline erosion due to subsidence and other factors (Glick et al. 2013). Wave exposure, duration of marsh inundation, dominant vegetation type, and hydrologic regime were considered in interpreting the influence of oiling or response actions on shoreline change and vegetation health metrics.

Injury observations for marsh habitats and associated fauna were quantified and expanded throughout the region using shoreline oiling classifications based on degree and duration of exposure (Nixon et al. 2016) and relationships with additional exposure metrics. For example, site-specific injury to plants was spatially extended across the region using the observed relationships between plant stem oiling (which was measured at each wetland injury study site) and shoreline oiling (which was characterized more broadly), weighted to account for bias in sampling designs (Nixon et al. 2015). Injury to marsh fauna (with the exception of periwinkles, which were only evaluated in one oiling category) was expanded using the relationship between observed marsh soil PAH concentrations (which were measured at each wetland injury study site) and shoreline oiling categories, also adjusted to account for sampling bias. Injury to fiddler crabs was described but not expanded to estimate the total length of shoreline injured. Estimates of the expected duration of injury to coastal wetland habitat were developed through a combination of trend analysis of field observations, and by applying information and experience from prior spills. Key datasets for determining and quantifying injury to marsh flora and fauna include coastal wetland vegetation health metrics and soil PAH concentrations measured annually from 2010 to 2013 (Hester et al. 2016; Rouhani et al. 2017, this Theme Section).

The assessment of injury to subtidal oysters *C. virginica* focused on the release of river water as part of response actions in the summer of 2010, as oysters are known to be intolerant of prolonged periods of low-salinity water (VanderKooy 2012). Exposure to oil in surface waters and on the shoreline was also evaluated. The abundance of subtidal oysters was evaluated over time at sites representing a variety of oiling and salinity influences (Powers et al. 2015). Exposure to excess freshwater was evaluated using interpolated and modeled salinity concentrations in summer 2010 (McDonald et al. 2015) and by comparing those concentrations to summer conditions during pre-spill baseline years (Rouhani & Oehrig 2015). An area of freshwater impact was identified for oysters (Rouhani & Oehrig 2015), and injuries were quantified using observations of oyster cover and abundance for each sub-region affected (Powers et al. 2015). Estimates of the magnitude and expected duration of injury to subtidal oysters were developed by considering reproductive implications of lost spawning stock and modeled larval transport (Grabowski et al. 2015, Powers et al. 2015).

Assessment of ecological injury to beach habitat focused on evaluating the impact of the combination of oiling and response actions. Beach injury was evaluated using shoreline oiling classifications (Nixon et al. 2016) and information on severity and duration of response actions (Michel et al. 2017). Literature and information from prior spills were used to determine relative degrees of injury which were extended over the larger region to quantify lengths and areal extent of beach habitats that were injured. Information from prior spills was also used to estimate recovery rates of beach habitats (Michel et al. 2017).

Assessment of injury to SAV focused on the intersection of oiling and/or response actions with known areas of SAV habitat. SAV habitats representing a range of shoreline oiling conditions in the Chandeleur Islands were sampled and evaluated using aerial imagery analysis to determine changes in cover between 2010 and 2012 (Kenworthy et al. 2017). Soil, sediment, and tissue samples were analyzed for PAH concentrations to evaluate exposure between 2010 and 2011 (Cosentino-Manning et al. 2015). Recovery times for SAV habitat were estimated using data from injury assessments from prior vessel groundings (Kenworthy et al. 2017).

Results from studies for each nearshore habitat and species were analyzed using various univariate statistical tests. These tests were employed to examine whether sites exposed to oil or response actions differed from reference conditions. Multivariate and exploratory methods were also used to examine relationships between variables. For all analyses, a *p*-value of 0.1 was used to evaluate statistical significance of results and to balance Type I and Type II errors (Mapstone 1995). Observed effects to nearshore habitats and species were then integrated to evaluate the potential for ecosystem-wide impacts. Multiple indicators of injury evaluated for each habitat (coastal wetland, oysters, sand beaches, and SAV) were compiled by shoreline oiling categories to evaluate broader implications. These indicators were also used to determine which ecosystem services were likely affected by oiling and response actions.

## FINDINGS OF THE ASSESSMENT

Injuries to nearshore resources occurred across multiple trophic levels (from plants to predatory fish) and connected habitats, including coastal wetlands, beaches, nearshore and subtidal oysters, and SAV as a result of direct oiling and response activities. Ecological functions that support the health and produc-

tivity of the larger Gulf of Mexico system have been degraded, including the availability of abundant prey; stable marsh platform structure; nutrient cycling, water filtration, and clean soil and sediment that promote growth and survival of biota; healthy submersed and emergent plant cover that supports higher trophic levels and provides refuge from predators; and substrate for oyster spat settlement.

Multiple lines of evidence of an ecosystem level injury were developed through the application of interconnected conceptual models and testable hypotheses. Pathways for oil to reach nearshore habitats were identified and confirmed using observations of stranded oil, remote sensing data, and chemical analysis of stranded oil, soil, and sediment, including forensic analysis to verify the presence of oil released from the well. Analysis of this evidence verified that oil entered the nearshore environment on the surface of the water, driven by winds and currents. As it reached the shore, oil became entrained in surf as droplets or attached to particles, stranded on vegetated and beach shorelines, and redistributed to nearshore subtidal areas (Fig. 1). Over time, residual oil and components were found on plants, in beach sands and marsh soils, and incorporated into submerged sediments (Rouhani et al. 2017).

Exposure to oil released from the well occurred over a broad area and was long lasting. Each of the nearshore ecosystem habitat types in the northern Gulf of Mexico was oiled to varying degrees as a result of the spill, and the linear geographic extent of shoreline oiling was the largest of any marine spill globally (Nixon et al. 2016). Oil was observed on more than 2113 km (1300 miles) of shorelines from Texas to Florida. At least 965 km (600 miles) of beaches and 1105 km (687 miles) of coastal wetland shorelines were oiled (Nixon et al. 2016).

PAH concentrations were measured in soil, sediment, and biological tissues to provide additional information on exposure to oil released from the well. Soil from oiled coastal wetland habitats sampled between 2010 and 2013 contained elevated PAH concentrations when compared to unoiled shorelines, and when compared to ambient concentrations calculated using samples that were unlikely to contain DWH oil (as confirmed by forensic analysis; Rouhani et al. 2017). Concentrations of PAHs in nearshore submerged sediments were also elevated along oiled shorelines, especially within 50 m of shore (Rouhani et al. 2017). PAH concentrations in invertebrate tissues collected from 2011 to 2012 from beach and coastal wetland shoreline areas were variable and showed no apparent trend with the degree

Table 1. Summary of injuries to oiled marsh vegetation and fauna. Metrics were evaluated by comparing oiled sites to un-oiled reference conditions. Regional expansion was completed using shoreline oil characterization (Nixon et al. 2016). Recovery times were estimated using field observations and information from prior spills. NC: not calculated

Model species/ injury metric	Maximum % change relative to reference	Zones of injury	Km (miles) of shoreline affected	Observed time period of injury	Expected recovery time (yr)
Mainland herbaceous live above-ground biomass <sup>a</sup>	45	Edge, interior	>563 (>350) <sup>b</sup>	2010–2013	2–8 <sup>c</sup>
Mainland herbaceous total live cover <sup>a</sup>	44	Edge, interior	>563 (>350) <sup>b</sup>	2010–2013	2–8 <sup>c</sup>
Amphipod survival <sup>d</sup>	95	Edge, interior	249 (155)	2010–2013	>4
Periwinkle abundance <sup>e</sup>	90	Edge, interior	62 (39) <sup>d</sup>	2011	>10
White shrimp growth (oil) <sup>d</sup>	46	Intertidal, edge	288 (179)	2011	>2
Brown shrimp growth (oil) <sup>d</sup>	56	Intertidal, edge	288 (179)	2011	>2
<i>Fundulus</i> hatch success <sup>d</sup>	99	Edge	62 (39)	2010–2013	>4
Flounder growth <sup>d</sup>	90	Edge	62 (39)	2011–2013	>3
Red drum growth <sup>d</sup>	47	Edge	62 (39)	2010–2012	3
Fiddler crab burrow density <sup>f</sup>	39	Edge, interior	NC	2010–2014	>4
Nearshore oyster cover <sup>g</sup>	99.5	Intertidal	250 (155)	2012–2013	No recovery

<sup>a</sup>Hester et al. (2016), except as noted  
<sup>b</sup>Nixon et al. (2015)  
<sup>c</sup>Michel & Rutherford (2014)  
<sup>d</sup>Powers & Scyphers (2016)  
<sup>e</sup>Zengel et al. (2016a), except as noted. See also Zengel et al. (2017, this Theme Section)  
<sup>f</sup>Zengel et al. (2016b)  
<sup>g</sup>Powers et al. (2017, this Theme Section)

of shoreline oiling (Oehrig et al. 2015). PAHs in SAV plant tissue and invertebrate whole bodies collected from SAV habitats were higher in oiled areas in August and September 2010 than in samples collected in June 2010, and declined to concentrations similar to pre-spill conditions by June 2011 (Cosentino-Manning et al. 2015). All data collected as part of the DWH NRDA are available online.<sup>1</sup>

NRDA results are generally consistent with those found in other studies after the spill, where concentrations of oil components were elevated in wetland soil (Keevan 2012, Lin & Mendelssohn 2012, Turner et al. 2014, Atlas et al. 2015), submerged sediment (Floyd et al. 2012, Sammarco et al. 2013), nearshore surface water (Allan et al. 2012, Sammarco et al. 2013), and biological tissues (Xia et al. 2012, Sammarco et al. 2013). Two other studies did not find evidence of oil accumulation in oysters or other filter feeders after the spill (Carmichael et al. 2012, Fry & Anderson 2014). Some oil components were observed to be elevated in marsh soils up to 3 yr after the spill (Keevan 2012, Turner et al. 2014). Oil-

degrading bacteria in marsh soils responded positively to the presence of oil (Beazley et al. 2012, Natter et al. 2012, Atlas et al. 2015).

Oiling caused multiple injuries to coastal wetland habitats, including reductions in aboveground biomass and total plant cover in mainland herbaceous salt marshes. Herbaceous salt marsh vegetation in Louisiana exposed to more than trace oiling of plant stems exhibited evidence of injury, particularly in the marsh edge zone closest to the shoreline (Hester et al. 2016). More than 1105 km (687 miles) of coastal wetland shoreline were reportedly oiled throughout the Gulf (Nixon et al. 2016). Injury was estimated to occur over at least 563 km (350 miles) of shoreline in Louisiana mainland herbaceous salt marsh alone, based on the extent of observed shoreline and plant oiling conditions (Nixon et al. 2015, 2016) and the findings of injury to oiled mainland herbaceous plants (Hester et al. 2016). NRDA findings of injury to wetland vegetation cover, photosynthesis, and biomass, particularly along the marsh edge (Table 1), were consistent with those found by other researchers (Lin & Mendelssohn 2012, Mishra et al. 2012, Wu et al. 2012, Khanna et al. 2013, Kokaly et al. 2013, Shapiro et al. 2016). Plant stem oiling (where oiling occurred over more than 90% of the length of the stem), soil and shoreline oiling, associated response actions, and loss of nearshore oyster cover

<sup>1</sup>DWH NRDA data are available at <https://www.doi.gov/deepwaterhorizon/adminrecord>; <https://pub-dwhdatadiver.orr.noaa.gov/dwh-ar-documents/902/DWH-AR0305139pdf>; <https://pub-dwhdatadiver.orr.noaa.gov/dwh-ar-documents/858/DWH-AR0305135.pdf>



also affected the physical stability of vegetated shorelines, with an increased erosion rate observed between 2010 and 2013 compared to reference conditions (Gibeaut et al. 2015, Roman 2015, Silliman et al. 2016, Powers et al. 2017). Increased erosion of oiled vegetated shorelines is estimated to have occurred over at least 174 km (108 miles) (Powers et al. 2017). Other researchers also reported increased rates of shoreline erosion after oiling (Silliman et al. 2012, McClenachan et al. 2013, Zengel et al. 2015, Lin et al. 2016). These injuries to coastal wetland vegetation have significant implications for the overall health and productivity of the Gulf system by reducing primary production of the marsh and the stability of the marsh platform. Marsh plants form the basis of food webs and release nutrients through photosynthesis and decomposition (Peterson & Howarth 1987). Marsh vegetation can also remove excess nutrients from runoff from upland areas (Fisher & Acreman 2004). Marsh habitat (particularly the marsh edge, where the most severe injuries occurred) supports the productivity of open water areas by facilitating movement of animals and nutrients between emergent vegetation, intertidal, and subtidal areas for forage and refuge (Levin et al. 2001), and its degradation or permanent loss will have significant consequences for productivity of fish and wildlife.

In addition to injuries to the vegetation, all marsh faunal species evaluated in the most heavily oiled areas showed evidence of injury. Examples of observed or predicted injury include a reduction in periwinkle *Littoraria irrorata* abundance and recruitment (Zengel et al. 2016a; Zengel et al. 2017, this Theme Section; S. Pennings et al. unpubl.); reductions in growth (and resulting reduced survival) of shrimp (*Farfantepenaeus aztecus*, *Litopenaeus setiferus*), juvenile southern flounder *Paralichthys lethostigma*, and red drum *Sciaenops ocellatus* (Powers & Scyphers 2016); reduced amphipod survival (Powers & Scyphers 2016); reduced hatching success of Gulf killifish *Fundulus grandis* (Powers & Scyphers 2016); reduced fiddler crab (*Uca* spp.) abundance (as measured by burrow density, Zengel et al. 2016b); and decreased cover of nearshore oysters *Crassostrea virginica* (Powers et al. 2017) (Table 1). Abundance and cover of nearshore oysters did not appear to be affected by the release of river water during response actions (Powers et al. 2017). Supporting laboratory toxicity tests indicate that the faunal effects described here could have arisen through a combination of chemical toxicity and fouling or smothering (Morris et al. 2015). Faunal injury was expanded over

at least 62 km (39 miles) of mainland herbaceous salt marsh shoreline based on the linear extent of observed shoreline oiling where PAH concentrations exceeded toxic levels in laboratory studies or where injury was detected in field studies (Nixon et al. 2016, Powers & Scyphers 2016, Rouhani et al. 2017). Bird injury was also documented; pelicans and other colonial birds nesting at 2 colonies in Barataria Bay declined by 48% between May and June, 2010, which was attributed to extensive oiling of marsh habitat (Baker et al. 2015). Non-NRDA studies also observed potential injury to Gulf killifish, benthic foraminifera, fiddler crabs, and terrestrial arthropods in oiled salt marshes (Whitehead et al. 2012, McCall & Pennings 2012, Hatch 2013, Pennings et al. 2014).

In addition to the effects of marsh oiling on fauna, summer river water releases as part of spill response likely reduced benthic prey abundance (Adamack et al. 2012) and could have further reduced production of juvenile shrimp and other marsh fauna. Adamack et al. (2012) modeled the effects of a late April/May water release and predicted that benthic production would be 40 to 60% less than under baseline conditions. Rose et al. (2014) suggested that estuarine-dependent fish and invertebrates would be displaced by river water released under an 'oil spill' scenario (similar to those that occurred after the DWH spill), but they did not quantify the effects in terms of lost production.

Injuries to marsh habitat have larger implications for the nearshore ecosystem as adverse effects to one species are likely to cascade through the food web. For example, *F. grandis* represent a prey item for wildlife (such as wading birds) and many sport fish, including flounder and speckled trout (Ross 2001). Some fauna play an important role in sustaining a healthy marsh. Fiddler crabs modify sediments, organic material, and nutrient cycling through their burrowing and feeding activities, affecting marsh soil communities and vegetation (Montague 1982, Hoffman et al. 1984, Gittman & Keller 2013). Marsh periwinkle grazing plays a key role in cycling organic matter and nutrients, influencing marsh food webs and marsh plant productivity (Kemp et al. 1990, Silliman & Zieman 2001). Marsh plants and nearshore oysters can dampen wave energy, trap and stabilize soil and adjacent sediment, and provide structure and cover for predators and prey (Zimmerman et al. 2000, Piazza et al. 2005, Mudd et al. 2010, Powers et al. 2017). Dolphins using Barataria Bay were also evaluated as part of the NRDA. Dolphins captured and evaluated in 2011 were 5 times more likely to have moderate to severe lung disease when com-

pared to Sarasota Bay dolphins (Schwacke et al. 2014). Continued monitoring of the Barataria Bay population in 2013, 2014, and 2015 indicated that survival and reproduction were also lower than in the reference population (Lane et al. 2015). The combination of injury to vegetation, shoreline stability, and marsh fauna (Table 1) indicated that services the habitat provides to humans and other ecosystem components was likely reduced.

Subtidal oysters were evaluated separately from nearshore oysters due to their greater distance from the marsh edge. Low-salinity conditions in the summer of 2010 resulted in mortality of an estimated total of 1.2 to 3.3 billion market-sized equivalent subtidal oysters over 483 km<sup>2</sup> in Barataria Bay and 362 km<sup>2</sup> in Black Bay/Breton Sound, respectively (Powers et al. 2015). In contrast to nearshore oysters (which were affected by shoreline oiling), no relationship between subtidal oyster abundance and shoreline or surface water oiling could be discerned (Powers et al. 2015). The combined loss of billions of nearshore and subtidal oysters resulted in failed recruitment over several years in the most heavily impacted areas (Barataria Bay, Black Bay/Breton Sound, and Mississippi Sound), with a total estimated loss of 4.0 to 8.4 billion market-sized equivalent oysters (Grabowski et al. 2015, Powers et al. 2015). The loss of nearshore and subtidal oysters likely impaired the ecological services that oysters typically provide, including supporting a robust commercial fishery. Oysters play an important role in the nearshore ecosystem in that they filter large volumes of water, recycle nutrients, and provide forage and refuge for other invertebrates and fish (Coen et al. 2007, Grabowski & Peterson 2007, Kellogg et al. 2013).

Injury to estuarine fish and invertebrates under surface oil slicks was assessed using a modeling approach (Travers et al. 2015). The observed extent and duration of surface slicks behind the barrier islands was calculated using synthetic aperture radar image analysis over time. Toxicity tests were conducted using slicks of weathered oil from the spill, estuarine fish embryos and larvae, and juvenile estuarine invertebrates. Based on the range of sensitivities to oil and UV light observed in these tests, the trustees estimated a total mortality of 4 to 6% of larval fish and juvenile invertebrates over an average 2.5 m water depth for the area and time period of slick coverage (Travers et al. 2015). As part of a non-NRDA study, Moody et al. (2013) evaluated nekton utilization of salt marsh habitats in coastal Alabama before and after the spill. Significant differences in the recruitment of nekton were not observed in 2010

and 2011 when compared to 2009 (Moody et al. 2013), but the authors recognized that other areas of the northern Gulf received more intense oiling.

Of the 965 km (600 miles) of beaches with observed oiling, a total of 683 km (424 miles) were injured from oiling and disturbance from associated response actions (Michel et al. 2017). Repeated cleanup actions to remove oiled sand and wrack disrupted foraging for birds and nesting for birds and sea turtles (Cacela & Dixon 2013, Michel et al. 2017). Based on a literature review of the effects of human disturbance to nesting birds and response records, Ritter et al. (2015) concluded that response actions would have resulted in extensive nest failure for beach-nesting species over multiple nesting seasons. Because sand beaches and wrack support a variety of invertebrates that serve as important prey for migratory birds, fish, and other wildlife, physical disturbance associated with cleanup activities and oil can have serious implications for the larger system of connected habitats. Injured beaches in proximity to marsh habitats are particularly perilous for bird species that nest in dunes and feed in nearby oiled marsh habitats (Michel et al. 2017). In addition, loggerhead sea turtle *Caretta caretta* nest densities in northwest Florida were lower than expected in 2010 when compared to years before and after the spill. The trustees estimated that approximately 251 loggerhead nests were lost due to DWH response activities on nesting beaches (Cacela & Dixon 2013).

SAV was injured by oiling and response actions. Chandeleur Islands seagrass was particularly affected, with more than 109 ha (270 acres) destroyed (Kenworthy et al. 2017). Chandeleur Islands seagrass habitat is unique in its extent and ecological connection to the wider Gulf of Mexico, representing one of the largest areas of seagrass beds between Pensacola Bay, Florida, and Laguna Madre, Texas (Handley et al. 2007). Seagrass meadows of the Chandeleur Islands provide forage and refuge for birds, sea turtles, and pelagic juvenile fish and invertebrates and trap and stabilize sediment (Kenworthy et al. 2017). Loss of this habitat, therefore, has broader implications for the stability of the Chandeleur Islands system, which is slowly migrating westward. Sediments deposited on the back barrier shelf are trapped and stabilized by seagrasses which help maintain the islands' elevation above sea level. The loss of seagrasses disrupts this important service and could accelerate the physical degradation of the islands (Kenworthy et al. 2017).

Injuries to SAV habitats from propeller scars generated by response vessels were documented in many

coastal areas from the Chandeleur Islands to Apalachee Bay, Florida (Meehan 2015). Freshwater SAV habitats in Jean Lafitte National Historic Park and Preserve were altered by release of river water as part of response actions (Weston Solutions Inc. 2015). These habitats also provide important forage and refuge habitat for birds and wildlife.

Long-lasting ecosystem-level injury is particularly likely where multiple injured habitat types occur in close proximity and the most severe shoreline oiling and response actions occurred. Marsh, oyster, and beach habitats injured by a combination of oil, physical response actions, and releases of river water occur in close proximity in Barataria Bay, Black Bay/Breton Sound, and Mississippi Sound. Beach, coastal wetland, and SAV habitats in the Chandeleur Islands were also injured by oiling. Unless shoreline is eroded, prior studies indicate that oiled vegetation may recover within 2 to 8 yr based on the warmth of the Gulf of Mexico climate, the variability of Gulf shoreline oiling, and the characteristics of the spilled oil (Michel & Rutherford 2014, Nixon et al. 2015). Injury to marsh fauna will persist until soil concentrations drop below concentrations observed to be toxic in lab studies, or until long-lived species such as periwinkles can recover their population structure, which could be more than 10 yr in the most heavily oiled areas (Powers & Scyphers 2016, Zengel et al. 2016a, 2017). Recovery of predators that rely upon amphipods and other sensitive species will be delayed even further. Marsh edge erosion and destruction of nearshore oyster cover will be permanent and can only be addressed through active intervention or restoration (Powers et al. 2017). Subtidal oyster recruitment may slowly recover naturally over many years, or the spill-related losses may have been so severe that active restoration will be required to initiate recovery (Grabowski et al. 2015). The largest patches of SAV, which spread slowly through rhizome growth, will also take decades to recover (Kenworthy et al. 2017). For full ecosystem recovery to occur, environmental and habitat conditions must be suitable to support normal life history functions for all species that were affected.

### CONSIDERATIONS FOR FUTURE SPILLS

The assessment of injuries to nearshore habitats under the DWH NRDA illustrated the benefits of applying new methods and integrated assessment approaches. Although very large oil spills are rare, lessons from the evaluation of multiple lines of evi-

dence of injury to nearshore habitats and resources will allow trustees to improve future assessments of oil spills, regardless of the size and scope.

There is increasing interest in evaluating ecosystem-level injury from pollution incidents in the context of the Millennium Ecosystem Assessment (MEA 2005). The DWH NRDA illustrates that assessing injuries to ecosystem services can be accomplished within the requirements of NRDA if ecosystem services can be clearly defined and tied to measurable or observable indicators that respond to oil exposure. Carpenter et al. (2009) recommended general principles for evaluating ecosystem services that can also be applied to NRDA, including associating study designs to specific research questions within an overarching conceptual framework; applying models that bridge observations to concepts and theories; and contrasting injuries across locales, scales, and time periods. The use of integrated conceptual models in the DWH NRDA provided a framework for evaluating injury on an ecosystem scale. To build preparedness for future spills, trustees can adapt the DWH NRDA conceptual models to appropriate locations for likely spill scenarios. Describing pathways of exposure and injury for beaches and marshes in advance would expedite implementation of studies when spills occur. Integrating the application of the models as much as possible across nearshore habitats will allow trustees to identify (and possibly quantify) injuries to the ecosystem services described by the MEA (2005): provisioning, regulating, cultural, and supporting services. These are defined by the benefits people obtain from ecosystem attributes and components. Provisioning services include food and water; regulating services include flood and disease control; cultural services provide spiritual, recreational, and cultural benefits; and supporting services include nutrient and water cycling that maintains the conditions for life on Earth (MEA 2005).

NRDA sampling needs are typically scaled based on the size and specific conditions of the spill and requirements for selecting appropriate compensation and restoration. Many small spills are assessed using minimal information (shoreline oiling observations, samples to confirm the source of the oil, and literature on likely effects). For larger and more complex spills (with DWH being an extreme example of these), evaluating species and life stages intimately associated with shoreline oiling and that represent multiple feeding guilds and trophic levels allows trustees to integrate indicators of habitat injury with greater certainty. In the DWH NRDA, assessing multiple species and life stages found in oiled coastal

wetland habitats illustrated larger ecosystem implications of injury. Co-locating sampling stations for assessment of soil chemistry, nearshore sediment chemistry, vegetation health, shoreline erosion, and marsh fauna abundance over transects from upland to subtidal environments maximizes the ability to compare and connect multiple injuries and scale and plan restoration to compensate for losses. For example, in the DWH NRDA, co-locating nearshore oysters with marsh vegetation injury sampling locations revealed service losses (indicated by marsh erosion) that would not otherwise have been apparent. Timing of studies is also important. For example, if bioaccumulation of PAHs and food web exposure is a concern, tissue samples from organisms with limited capacity to metabolize these compounds should be collected as soon as possible after shoreline oiling occurs.

Large, complex, or contentious spills often require multiple lines of evidence from a combination of field observations, laboratory studies, and literature findings in order to satisfy NRDA requirements to connect pathways, exposure, and injuries. For example, in the DWH NRDA, combining field studies of periwinkle *Littoraria irrorata* abundance in oiled areas with laboratory studies to evaluate periwinkle survival and behavior after oiling provided compelling evidence of a causal link between exposure and injury (Garner et al. 2015, Zengel et al. 2016a). Innovative field and laboratory methods for evaluating sensitive endpoints were developed and applied to NRDA's for the DWH and other recent spills. For example, as part of the NRDA for the 2007 Cosco Busan oil spill in San Francisco Bay, California, caged herring *Clupea pallasii* eggs were deployed in the field to examine abnormalities associated with proximity to oiled shorelines (Incardona et al. 2012). After the DWH spill, short-term growth was measured in juvenile shrimp (*Farfantepenaeus aztecus*, *Litopenaeus setiferus*) caged adjacent to oiled shorelines. That study evaluated combined pathways of exposure, including nearshore sediment and associated prey and runoff of oil, oiled soil particles, and detritus from adjacent marsh surfaces (Rozas et al. 2014). This field-based design provided a more realistic evaluation of the implications of shoreline oiling than a lab study that only exposed animals to contaminated sediment. Caged animal approaches should be further expanded to develop methods for additional species and regions. For the DWH NRDA, conducting laboratory toxicity testing of sediment spiked with weathered oil over the gradient of PAH concentrations observed in field samples allowed for com-

parison of injury metrics across species and across the region using a standardized approach. These techniques could be further developed to evaluate sensitive sub-lethal endpoints for fish and invertebrates exposed to oil. After the DWH spill, high-resolution aerial imagery proved useful in evaluating shoreline erosion in Barataria Bay and changes in Chandeleur Islands SAV cover over time. In the future, it may be possible to gather appropriate imagery using unmanned aircraft systems with less time and expense.

NRDA regulations also require trustees to evaluate injury to natural resources in comparison to baseline conditions. Because pre-spill data on target organism abundance and injury endpoints for fish and invertebrates are so infrequently available, the selection of reference sites is especially important in relating injury to exposure, as is the implementation of a robust stratified random sampling design. As applied in the DWH NRDA, the selection of reference sites and collection of supporting data should account for possible confounding factors such as wave exposure, dominant plant cover (habitat type), hydrologic regime, and inundation (including the magnitude, frequency, and duration of inundation). In this spill, the loss of edge stations due to erosion reduced the ability to observe differences between oiled and reference sites over time. Baseline erosion rates should be considered at future spills when conducting power analysis to determine sampling requirements to test hypotheses. To determine whether PAH concentrations are elevated at oiled sites when pre-spill data are not available, it is possible to derive ambient concentrations after the spill using forensic chemistry data to identify samples not influenced by the spill. These concentrations may vary considerably by sub-region depending on proximity to other sources (Rouhani et al. 2017).

There are several common approaches trustees use to meet the requirement of the NRDA regulations to quantify injury and translate injury metrics into restoration. Habitat equivalency analysis (HEA, Dunford et al. 2004) and resource equivalency analysis (REA, Snyder & Desvousges 2013) equate injured ecosystem services or resources with services or resources provided by restoration projects. HEA and REA are most commonly used in NRDA when restoration techniques are available that will directly replace lost services or resources. Value-to-cost approaches are less frequently used, but can identify restoration needed to compensate for lost services when requirements for HEA or REA are not met. In these techniques (e.g. contingent valuation,

Portney 1994), the monetary value of injuries (and/or services lost) is equated to the cost of restoration to compensate for the injuries (lost services). In all of these approaches, ecosystem services affected by the spill, and the ecosystem service benefits available through restoration, should be clearly understood and articulated.

In conducting NRDA, trustees typically simplify ecosystem services to those supporting primary and secondary production. By collecting broad and consistent information on shoreline oiling and additional exposure data at point-locations (such as plant stem oiling and soil PAH concentrations) using a statistically robust stratified random design, injuries observed at specific locations can be expanded to estimate effects over a larger area, as demonstrated in the DWH NRDA. These observations can be quantified in terms of numbers or biomass of individual resources lost (as in REA) or ecological services over a certain area or shoreline length (as in HEA). Individual metrics that may be affected by oil (e.g. marsh vegetation cover) can be associated with multiple ecosystem services (e.g. supporting, provisioning, and regulating), yet trustees can only claim compensation for the same injury once. Trustees must also account for baseline conditions and confounding factors that influence the ecosystem service being assessed.

Quantifying and valuing ecosystem services are challenging, partly because methods for analyzing and valuing many ecological functions and ecosystem services have not yet been developed (Carpenter et al. 2009). An individual ecosystem service may be provided through the combined influence of many specific resource components. For example, shoreline stability after the DWH spill was influenced by vegetation cover, above- and below-ground biomass, nearshore oyster structure, and geomorphology. The more services that are potentially affected by a spill, the more complex the assessment will be. No assessment, however, can fully examine and evaluate all ecosystem services. To support evaluation of broader ecosystem services as part of NRDA, techniques are needed to demonstrate how physical and chemical processes affect habitat structure and ecological function, and ultimately, how they contribute services over a larger area.

Finally, as demonstrated by the DWH NRDA, coordinating NRDA with spill response activities can result in efficiencies and benefits to both groups. Shoreline oiling observations collected as part of spill response actions provided initial information for evaluating exposure to nearshore habitats and

species, as well as consistent information to support expansion of injury across the region. While conducted to support cleanup decisions, these surveys provided broad spatial coverage and a consistent set of data. A combination of response data and additional data collected during early shoreline assessments (e.g. average plant stem oiling in each shoreline segment) facilitated selection of sampling strata that represented exposure conditions likely to be associated with injury to marsh vegetation and fauna (including selection of un-oiled reference sites). Although it is not required to distinguish between injury from oil and injury from response actions (since both are compensable under NRDA regulations), gathering appropriate information on response actions helps interpret observed effects and address possible confounding factors. Coordination between response and NRDA activities can improve response to future spills, since NRDA assessment findings can illustrate ecological trade-offs of shoreline and offshore response actions.

Based upon observations of the DWH NRDA, Peterson et al. (2012) provided a number of recommendations to improve NRDA, especially for spills associated with deepwater well blowouts. They correctly identified many constraints on NRDA associated with provisions of OPA, including the requirement to rely on well-established scientific approaches, the requirement to collaborate closely with responsible parties, and a focus on restoration of resources of high known value to the public. Program funding limitations and the OPA definition of restoration (replacing, restoring, or acquiring the equivalent of injured natural resources) limit opportunities for research not directly tied to quantifying injury and planning restoration for specific spills (Peterson et al. 2012). The disturbance associated with some cleanup actions on marsh and beach shorelines may have exacerbated or prolonged ecological injury and recovery. However, for the DWH spill, trustees were able to determine and quantify injuries from response actions (especially the effect of river diversions on oysters and impacts from beach cleanup activities). It is unclear whether the use of dispersants resulted in less cohesive surface water oiling and less intense shoreline oiling, as theorized by Peterson et al. (2012). Although it may not be possible to determine the influence of the use of dispersants on the extent and magnitude of shoreline oiling during this spill, contrasting the scope and magnitude of shoreline injury with predictions of mortality to early life stage fish and invertebrates in surface waters provides a basis for



evaluating some of the benefits and drawbacks of dispersant use, and additional research and analysis of the trade-offs would further inform future spill response and damage assessment priorities.

## CONCLUSIONS

For the DWH NRDA, shoreline oiling characterization proved a useful indicator of exposure for nearshore organisms. Injury was observed in all oiled nearshore habitat types examined, including coastal wetlands, beaches, SAV, and oyster reef areas. Multiple pathways and multiple mechanisms of impact were apparent, including chemical toxicity, smothering/physical fouling, physical disturbance, and intolerance of excess freshwater (low salinity).

Injury was quantified in Louisiana mainland herbaceous marshes that received even a trace amount of shoreline oiling. Both plant stem oiling and soil oiling were associated with injury, which included loss of plant cover; reductions in above-ground biomass; reduced abundance and/or growth of periwinkle snails *Littoraria irrorata*, fiddler crabs, shrimp (*Farfantepenaeus aztecus*, *Litopenaeus setiferus*), fish (*Paralichthys lethostigma*, *Fundulus grandis*, *Sciaenops ocellatus*), and nearshore oysters *Crassostrea virginica*; and accelerated erosion of marsh edge habitat. It was not possible to separate effects of oiling from effects of response actions in all instances. It is likely that freshwater releases also reduced production of benthic invertebrates and their predators. The marsh edge, which is the most productive zone in many respects and provides transient faunal access to flooded marsh surfaces for refuge and foraging (Zimmerman et al. 2000), was the most severely oiled and most severely injured. Because these areas promote rapid growth of juvenile fish and invertebrates of commercial importance and are also used by numerous bird species, other wildlife, larger finfish and crabs, and coastal dolphins, loss and degradation of this habitat will have broader implications for the ecosystem. Marsh recovery is expected to take more than 10 yr for long-lived species such as periwinkles (Zengel et al. 2016a), while eroded shoreline has been permanently lost (Silliman et al. 2016, Powers et al. 2017).

Hundreds of kilometers of beaches were injured by oil and response actions. Billions of subtidal oysters perished due to the release of river water as part of response actions, and oyster recruitment was affected over a large area. SAV habitats in the Chandeleur Islands have also been degraded, and natural

recovery of the largest patches of seagrass is likely to require more than 20 yr.

Ecosystem services affected by the spill include supporting services such as primary production and provisioning services such as fish and shellfish abundance. Although not directly measured, regulating services associated with stable marsh environments, such as coastal storm and flood protection, may also have been disrupted by the increased shoreline erosion associated with the spill. The loss of billions of oysters will reduce nutrient cycling and water filtration services.

The approaches and methods used in the DWH NRDA will improve the ability of trustees to evaluate integrated ecosystem impacts of oil spills of any size. Advances and innovations included the broad and integrated use of:

- unified conceptual models to understand pathways for oil and response actions to reach and injure nearshore habitats;
- soil PAH concentration measurements, forensic evaluations of soil and sediment PAH patterns, toxicity tests to determine lethal and sublethal PAH concentrations to resident species, field evaluations of vegetation health, marsh faunal abundance, shoreline change, and nearshore oysters at the same locations over time;
- evaluation of oyster recruitment success over a broader region through time; and
- high-resolution aerial imagery to evaluate shoreline change and SAV cover.

Trustees can improve future oil-spill NRDA efforts by planning ahead to develop general conceptual models to support assessment of marsh, beach, SAV, and oyster habitats. Integrating assessments using co-located transects and similar approaches and endpoints across habitats will assist in evaluating larger-scale ecosystem-level injuries. Developing clear, testable hypotheses using shoreline oiling characterization as an exposure framework for nearshore species and habitats (when combined with data on plant oiling and PAH concentrations in soils and sediments) will allow conclusions of individual studies to be extended to broader areas of oiled shorelines. A combination of field studies and laboratory toxicity testing can help elucidate relationships between exposure and injury. New and expanded techniques are needed, as are methods for evaluating additional ecosystem services within the constraints and requirements of NRDA. Integrating NRDA and response actions and further exploration of response option trade-offs will benefit both efforts.

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