Oil Contamination in Mississippi Salt Marsh Habitats and the Impacts to Spartina alterniflora Photosynthesis

Patrick D. Biber, Wei Wu, Mark S. Peterson, Zhanfei Liu, and Linh Thuy Pham

CONTENTS
Introduction ................................................................................................................................. 134
Methods........................................................................................................................................ 138
Study Sites........................................................................................................................ 138
Sampling of Sediments, Plants, and Animals for Oil Contamination ......... 140
Comparison of Marsh Oil with Macondo Oil ............................................................. 141
Plant Stress Responses .................................................................................................... 141
Pulsed Amplitude Modulation Chlorophyll Fluorescence ........................... 142
LI-COR Chlorophyll Fluorescence and Gas Exchange .................................. 143
Statistical Analyses .......................................................................................................... 143
Results........................................................................................................................................... 144
Spatial Contamination ................................................................................................... 144
Time-Series Contamination ......................................................................................... 144
Photosynthesis Responses to Oil Contamination ....................................................... 150
Pulsed Amplitude Modulation Chlorophyll Fluorescence
at Marsh Point ........................................................................................................ 150
LI-COR Fluorescence and Gas Exchange at Marsh Point ........................................ 151
One-Year Assessment ................................................................................................. 155
Discussion ......................................................................................................................... 155
Contamination ................................................................................................................. 155
Location-Specific Oil Impacts ..................................................................................... 159
Acute and Chronic Photosynthesis Responses .................................................. 161
Conclusions ...................................................................................................................... 164
Acknowledgments ............................................................................................................ 164
References........................................................................................................................ 164
Introduction

The Deepwater Horizon (DWH) explosion and subsequent oil spill in the northern Gulf of Mexico (GOM), from April 20 to July 15, 2010, are the largest accidental marine oil spill in the history of the U.S. petrochemical industry (Read 2011). This accident released about 4.9 million barrels \((7.0 \times 10^6 \text{ m}^3)\) of crude oil into the open ocean from the leaking Macondo (MC252) well at 1.5 km depth (Crone and Tolstoy 2010) and greater than approximately 170 km from the Mississippi mainland coast. Salt marshes are important coastal ecosystems because they are highly productive. They also provide valuable ecosystem services, such as the provision of food and shelter for many organisms (Turner 1977; Boesch and Turner 1984; Phillips 1987; Cai et al. 2000; Beck et al. 2001), carbon sequestration (Chmura et al. 2003), shoreline stabilization and storm protection (King and Lester 1995; Moeller et al. 1996), filtration of excess nutrients (Valiela et al. 2000; Tobias et al. 2001a,b; Valiela and Cole 2002), and valued recreational and aesthetic opportunities (Lee et al. 1992; Engle 2011; Jordan and Peterson 2012). Coastal wetlands are not only threatened by stressors from the terrestrial side \((\text{ sensu }\) Peterson and Lowe 2009), but are also at risk from ocean-side stressors that include pollutants such as oil.

Oil spills in the marine environment have a long history, with numerous studies following the acute and chronic impacts of oil and how they affect the recovery of various coastal habitats and species (Baca et al. 1987; Kenworthy et al. 1993; Duke et al. 1997; Peterson et al. 2001; Graham et al. 2010; Fordie and Heck 2011; Ortmann et al. 2012; Kolian et al. 2013). From this literature it is clear that the rate of recovery varies by habitat type largely as a function of coastline energetics (NOAA 2012), degree of direct exposure to oil experienced by subtidal versus intertidal organisms (Kenworthy et al. 1993; Peterson et al. 2001; Venosa and Zhu 2003), and mean annual temperature (Baca et al. 1987; Duke et al. 1997). In combination with these factors, the overall toxicity of oil decreases rapidly over time, with warmer conditions promoting more rapid “weathering” (Lee and Page 1997; Venosa and Zhu 2003; Aeppli et al. 2012; Mendelsohn et al. 2012).

The degradation of oil in the marine environment varies substantially as a function of temperature, oxygen content, nutrients, pH, and salinity (Venosa and Zhu 2003). Crude oil in water is broken down by important weathering processes, including spreading, evaporation, dissolution, emulsification, sedimentation, biodegradation (microbial oxidation), and photooxidation (Hunt 1996; Fingas 1999; Plata et al. 2008; Aeppli et al. 2012; Liu et al. 2012; Mendelsohn et al. 2012), all of which reduce the toxicity of the oil over time. In addition, the GOM is widely recognized as having a native microbial biota that is adapted to consuming hydrocarbons as an energy source, in part coming from extensive natural seeps on the seafloor, and in part a response to nearly a century of oil and gas extraction activities (Lin and Mendelsohn 1998; Venosa and Zhu 2003; Hazen et al. 2010; Orcutt et al. 2010; Edwards et al. 2011; Bik et al. 2012).

Oil impacts along the northern GOM coast in 2010 were documented extensively (e.g., daily shoreline cleanup assessment technique maps and daily Administration-Wide Response to DWH oil spill press releases) and where feasible (e.g., sand beaches) oil was removed soon after arrival. In less accessible locations, such as salt marsh shorelines, oil was documented but generally left in place or received minimal cleanup actions to prevent further damage to the habitat. Silliman et al. (2012) reported that about 75 km of Louisiana salt marsh shoreline was affected by medium to heavy oiling, the most of any of the affected states. In Mississippi, small oil slicks (<100 m²) arrived sporadically and intermittently from early June 2010 through the remainder of the year, in fact, British
Petroleum (BP) contractors continued to monitor the arrival of heavily weathered oil during summer 2012 along barrier island beaches. The initial 2010 oil arrived onshore more frequently and as larger patches around the time tropical systems were active in the northern GOM, primarily because of storm surge and onshore winds. Oil impacts to salt marshes were generally restricted to a narrow ribbon (5–15 m wide) right along the coastline, with little to no penetration of oil into the marsh interior (Mendelsohn et al. 2012; Silliman et al. 2012). This fringing zone is typically vegetated by smooth cordgrass (*Spartina alterniflora*) in the northern GOM, although in portions of Louisiana and Texas the black mangrove (*Avicennia germinans*) may also occur (McMillan and Sherrod 1986; Lloyd and Tracy 1901; Patterson et al. 1993). All these factors contributed to an emerging pattern of heavily weathered oil arriving sporadically in small quantities, impacting localized areas of shoreline, and not resulting in an extensive, simultaneous oiling event. This is atypical of previously studied large-scale oil spill disasters (Lee and Page 1997) that included massive volumes of oil released in a short time and near to shore from either shipwrecks, such as the *Torrey Canyon* (Southward and Southward 1978), *Amoco Cadiz* (Baca et al. 1987), and *Exxon Valdez* (Wolfe et al. 1994), or coastal oil processing facilities such as in Panama (Duke at al. 1997) and Kuwait (Kenworthy et al. 1993).

The response of salt marsh vegetation to weathered crude oil is complex and variable, ranging from short-term reductions in photosynthesis and rapid subsequent recovery to complete mortality and long-term wetland loss when the roots are killed (Pezeshki et al. 2000, 2001; Roth and Baltz 2009; Engle 2011). Oil can affect the plants directly by coating the leaves and blocking gas exchange through the stomata, chemical toxicity can disrupt plant–water relations or directly kill living cells, and thick oil on the sediment can reduce oxygen exchange with the atmosphere causing negative consequences for root health (Baker 1970; Pezeshki et al. 2000; Ko and Day 2004). Oil that smothers plants can also increase temperature stress, especially during summer, and in combination with reduced photosynthetic gas exchange, it will cause rapid and acute mortality in leaves and stems (Baker 1970; Lin and Mendelssohn 1996; Ko and Day 2004). The extent of the acute impacts to salt marshes varies with the amount and type of oil, the weather and hydrologic conditions at the time, the species of plant, season, soil composition, and any physical disturbance caused by cleanup activities (Lin and Mendelssohn 1996; Hester and Mendelssohn 2000; Pezeshki et al. 2000; Mendelssohn et al. 2012). For instance, in a study that compared oil impacts on photosynthetic gas exchange of two important U.S. Gulf Coast plant species, smooth cordgrass and black needlerush (*Juncus roemeriarius*), smooth cordgrass was shown to be more sensitive to partial oil coating than black needlerush (Lytle and Lytle 1987; Pezeshki and DeLaune 1993). In contrast, moderate oiling from the DWH oil spill had no significant effect on smooth cordgrass, but it significantly lowered live aboveground biomass and stem density of black needlerush in the Bay Jimmy area of northern Barataria Bay, Louisiana (Lin and Mendelssohn 2012). Furthermore, smooth cordgrass was shown to be more sensitive to oiling during the spring/summer growing season than during the predormancy or dormant season in winter (Pezeshki et al. 2000; Mishra et al. 2012). These factors notwithstanding marshes in the northern GOM region can be resilient to oil effects in the long term (2 years or longer), and some studies show complete recovery is attainable 4 years after a serious oil spill event (Mendelssohn et al. 1993; Silliman et al. 2012).

Oil can cause long-term chronic consequences in coastal wetlands when it becomes incorporated into the fine anaerobic sediments and persists for many years (Krebs and Tanner 1981; Alexander and Webb 1987; Lin and Mendelssohn 1998; Mendelssohn et al. 2012). The severity of these chronic impacts will be influenced by the factors
Impacts of Oil Spill Disasters on Marine Habitats and Fisheries in North America

aforementioned for acute impacts, as well as the amount of oil penetrating into the sediments, the physical nature of the coastline (i.e., high- or low-wave energy), and the composition of the plant community (Dicks and Hartley 1982; Peterson et al. 2003; Ko and Day 2004; Mendelssohn et al. 2012). Once oil penetrates into the sediments, recovery to reference conditions may take 3–4 years (Hester and Mendelssohn 2000) or longer (Bergen et al. 2000; Michel et al. 2009; Mendelssohn et al. 2012). Under extreme circumstances, recovery may never occur due to sediment removal (Baca et al. 1987; Gilfillan et al. 1995) or accelerated erosion after vegetation morality (Mendelssohn et al. 2012). In one study following a crude oil spill in Galveston Bay, oil concentrations in the sediment ranged between 5 and 51 mg/g, which significantly reduced growth of smooth cordgrass over 18 months. This reduction in the plants led to shoreline erosion that did not become evident until 16–32 months after the oil spill (Alexander and Webb 1987).

In another study, Silliman et al. (2012) reported that heavily oiled (>100 mg/kg polyaromatic hydrocarbons, PAH) wetlands in Louisiana resulted in plant mortality within less than 5 months and the subsequent loss of plant roots increased the rate of coastline erosion over 18 months compared to adjacent control sites that remained vegetated. The interplay of vegetation and geomorphic processes requires better understanding to properly assess the acute and chronic impacts of oil on salt marshes and determine their long-term resilience.

One approach commonly used to evaluate the impacts to an organism, population, or habitat from an injury (physical or chemical) is the impact-recovery model (Kirsch et al. 2005), which has its underpinnings in ecological succession theory. This conceptual model outlines an acute stress response immediately after an impact (e.g., an oil spill) as a reduction in the function of a process (e.g., photosynthesis), with gradual recovery back to preimpact function over time (Figure 7.1). In some instances recovery may be protracted, or may remain depressed compared to adjacent control sites, resulting in a chronic stress effect. In oil spills that impact soft-sedimentary shorelines, chronic impacts are often observed when oil is incorporated into subsurface sediments causing a persistent source of toxic chemicals that are slow to degrade under anaerobic conditions, resulting in ongoing exposure to the affected plants and animals. In the case of plants, this incessant exposure to oil at the roots can result in chronic depression of photosynthesis and a lack of recovery, compared to rates observed at unimpacted control sites. The impact-recovery model has been commonly employed in Before After Control Impact (BACI) designs and is also increasingly being applied in habitat equivalency analysis (HEA) as part of the valuation of lost ecosystem services (Underwood 1994; Unsworth and Bishop 1994; Dunford et al. 2004; Cacela et al. 2005). As HEA will be included in the development of compensatory mitigation projects arising from DWH impacts, the question of how much photosynthesis was reduced due to oil-induced stress is an important part of the overall evaluation.

Determining acute or chronic stress in plants can be accomplished by measuring photosynthesis (Schulze and Caldwell 1990; Krause and Weis 1991). As net primary productivity is also a measure of energy available to support ecosystem structure and function (Cardoch et al. 2002), photosynthesis can be used to relate the impacts of oil exposure to ecosystem health. However, photosynthesis is affected by factors other than oil stress, such as seasonal changes in temperature and light with greater photosynthesis during the summer growing season and less activity during the dormant winter season. There are two main techniques for measuring photosynthesis in emergent plants: chlorophyll fluorescence and gas-exchange rate (Schulze and Caldwell 1990; Rohacek and Bartak 1999; Maricle et al. 2007).
Oil Contamination in Mississippi Salt Marsh Habitats

These two approaches target different steps in the photosynthesis reaction, with chlorophyll fluorescence measuring conversion efficiency of photon energy to biochemical energy occurring in the electron transport chain of the light reactions at the very beginning of the photosynthesis process (Figure 7.2). In contrast, the incorporation of CO₂ measured during gas exchange is indicative of the carbon fixation (Calvin) cycle occurring in the dark reactions at the end of the photosynthesis process. Because of the difference in the process being measured, the two approaches should present similar patterns in response to stress, but they are not directly comparable. Furthermore, stress measured by the chlorophyll fluorescence technique may show more rapid recovery than stress measured by gas exchange, as plants tend to optimize light reaction chemistry more quickly than the downstream dark reactions (Figure 7.2).

The goals of this study were to determine the effect of oil contamination in Mississippi salt marsh plants and how oil may have affected photosynthesis of smooth cordgrass, the main species affected, over 1 year. More specifically, the objectives of this study were to (1) determine the location and spatial extent of oil contamination in Mississippi salt marsh habitats; (2) quantify the concentration of oil that was present in sediments, plant tissues, and animal tissues, and how it changed over the course of 1 year; (3) measure the effects of oiling on plant photosynthesis (measured by \( F_{v}/F_{m} \), CO₂ flux) to determine acute and chronic responses; and (4) assess the time to recovery of photosynthesis at two contrasting locations—an eroding coastline exposed to high wave and tide energy and a protected, depositional coastline with low energy.

FIGURE 7.1
Conceptual diagram of recovery following an impact relative to control levels for photosynthesis. A linear recovery is assumed over 12 months, although nonlinear responses could also be used.
Impacts of Oil Spill Disasters on Marine Habitats and Fisheries in North America

Methods

We contacted local, state, and federal agency personnel, as well as private citizens, to ascertain locations in Mississippi where oil had been documented to come ashore during the summer months of 2010. Only locations with documented impacts (photos, GPS coordinates) were selected for the oil contaminant sampling. We had two different goals when sampling for oil contamination. Our first goal was to determine the spatial distribution of residual oil contamination in salt marsh habitats when we sampled in fall (October–November 2010) along the Mississippi coastline. Our second goal was to determine the temporal change in contamination over 1 year (July 2010–July 2011) at selected locations.

Study Sites

To determine the spatial distribution of oil contaminants along the Mississippi coastline, we visited four locations: Grand Bay National Estuarine Research Reserve (GBNERR), Marsh Point (MP), a lagoon on Horn Island (HI), and Sand Bayou (SB) near Waveland, Mississippi (Figure 7.3). These locations covered the east to west extent of oil in Mississippi salt marshes, as well as mainland and barrier island coastlines. In each location, we sampled...
replicate quadrats (for contaminants) or replicate plants (for photosynthesis) at one control
and at least one oil-impacted marsh treatment. At one location (GBNERR), oil was no lon-
ger visible in the oiled treatment at the time of sampling, highlighting the need for prior
documentation during the acute phase of this event. A base map of the Mississippi coast-
line was obtained from Mississippi Geospatial Clearinghouse (MGC 2011) in NAD83 and
then converted to WGS84 UTM 16N projection and the sampling locations are indicated
thereon. Inset images were created to the same scale (1:800) from Google Earth and the
three replicate quadrats sampled for contamination within each treatment at each location
are indicted (Figure 7.3). Plant photosynthesis sampling occurred in the same general area
within each treatment, but was not necessarily exactly within the quadrats.

To determine the temporal change in oil contamination, we sampled two locations with
different energy coastlines: a high energy, exposed, and eroding coastline at MP and a
low energy, protected, and depositional coastline inside a lagoon at HI. Sampling at MP
occurred less than 2 weeks after the initial oiling in July 2010, in fall (October 2010), in
spring (March 2011), and 1 year (July 2011) after the initial oiling had occurred. Sampling at
HI was only done twice, November 2010 and August 2011, due to logistic issues prohibiting
us from access to the location during the “cleanup” phase.
Sampling of Sediments, Plants, and Animals for Oil Contamination

At each treatment within location, we haphazardly selected 10 m of salt marsh dominated by smooth cordgrass. Three replicate quadrats (1 m²) located no more than 5 m apart were placed along this 10-m transect. As smooth cordgrass is a fringing species in northern GOM salt marshes, quadrats were always within 1–3 m of open water, ensuring that we sampled where oil impacts were most likely to have affected vegetation. In each quadrat, all sediment and plant biomass samples were collected in duplicate, and immediately placed in labeled glass jars provided by the analytical laboratory. We always sampled the control treatment first and the heavily oiled treatment last, to avoid potential contamination of tools and samples. Control treatments were sampled as close as possible to oiled treatments within each location to minimize differences due to spatial position.

In addition to sample collection, the following data were gathered at each treatment within location as part of the chain of custody documentation: GPS coordinates of each quadrat, photos from the boat of the shoreline, and at least one overview and one close-up photo of each quadrat to document the condition of the plants and sediments, air temperature, and the time of collection (U.S. Central Standard Time). All samples were placed on ice immediately after collection then transferred to the analytical laboratory later that same day.

To obtain a representative sample of plant tissues within each quadrat, we harvested 20–30 smooth cordgrass stems near the base, cut them into pieces that fit into 1000-mL glass jars, and divided the sample equally between two duplicate jars. After removing the plant sample, it was easy to access the sediments in the quadrat. Using a stainless steel gardening trowel, we collected sediments to a depth of no more than 8–12 cm and filled two 250-mL glass jars about two-third full. Preference was given for shallow sediments with visible oil contaminant over deeper sediments, where possible. All hardware (trowel, plant cutters) were cleaned with acetone until all traces of oil were removed and then wiped down twice more with fresh paper towels. Afterward, a triple rinse with deionized water was used to minimize potential contamination of samples between quadrats and treatments. Only one of the duplicate samples was submitted for analysis, the second was retained as an archival sample.

We collected animal tissue samples from small resident killifishes (Fundulus spp.) and grass shrimp (Palaemonetes spp.). To obtain these species, a bag seine net (3.05 m long × 1.83 m tall, mesh size 3.17 mm) or a dipnet (0.46 m × 0.36 m, mesh size 3.17 mm) was run along the 10 m of shoreline in front of the quadrats at low tide, and all specimens were hand picked out of the catch. Target animals were placed in a sealed plastic bag and immediately kept on ice. We attempted to collect a minimum of 50 shrimp and 10 fish from the treatments within each location, but were not always successful despite up to 1 hour of intensive sampling at each treatment within location. Nekton samples were processed by removing the lateral muscle tissue of the fish and the tail muscle of the shrimp after removal of the carapace. All animal tissues from individuals collected from a treatment within location were pooled for a minimum weight of 3–5 g required for analysis of hydrocarbons. Each sample was frozen at −20°C in 7-mL glass scintillation tubes until analysis.

Analysis of oil range organics (ORO, C₁₉–C₃₆) in sediments and tissues was performed by gas chromatography–flame ionization detection (GC–FID) following EPA 8015C protocol, and analysis of PAH was performed by gas chromatography–mass spectrometry (GC–MS) following EPA 8270C protocol at Micro-Methods Laboratory Inc. (Ocean Springs, Mississippi). Results were reported in milligrams per kilogram (i.e., ppm) for each sample provided or “ND” if not detectable. Because of interference with plant structural
compounds, varying dilutions were required to stay within instrument detection limits
that otherwise may have affected the minimum reporting limits (MRL). Using these meth-
ods, detection limits were 10 ppm for ORO and 0.030 ppm for PAH (Douglas et al. 2007).
Hydrocarbon concentration data and MRL were transcribed from the analytical reports
into a Microsoft Excel© v. 2007 spreadsheet for each replicate quadrat within treatment at
each of the four locations.

Comparison of Marsh Oil with Macondo Oil

Samples collected at MP in July 2010 were also sent to the University of Texas, Marine
Science Institute to compare oil from a heavily impacted marsh treatment with the oil
originating from the MC252 well. The oil smothering the smooth cordgrass leaves was
scrapped carefully into a glass vial using a Teflon knife and frozen at −20°C until analy-
sis. MC252 reference oil was acquired from BP. The oil obtained from the surface of the
MP plants was normalized to total solvent-extractable materials (TSEM), the TSEM was
750 mg/g. The oil extraction followed the protocols of Wang et al. (2004). Briefly, about
1 g of sample was weighed and extracted five times with Dichloromethane (DCM) using
sonication. The extracts were combined and dried by filtering through a glass column
packed with anhydrous sodium sulfate. The DCM extract was concentrated by a rotovap
and exchanged with hexane. An aliquot of the concentrated extract was transferred into
a chromatographic column packed with activated silica gel and topped with anhydrous
sodium sulfate. After the columns were conditioned with hexane, the concentrated extract
was loaded into the column and eluted with hexane as the fraction for measuring satu-
rated \( n \)-alkanes, and subsequently eluted with benzene in hexane (50% v/v) as the fraction
for measuring aromatic hydrocarbons. For the MC252 oil, the crude oil was diluted with
hexane and filtered through a sodium sulfate column. The procedures for cleanup and
fractionation were the same as for the MP plant oil. Analysis for \( n \)-alkanes (C\(_8\)–C\(_{40}\)), prista-
tane (Pr), and phytane (Ph) was performed on GC–FID, whereas PAHs were analyzed with
GC–MS in a selective ion mode.

Plant Stress Responses

To ascertain the impact of oil contamination on plant photosynthesis and time to recovery
to preoiled performance, we sampled plants repeatedly at MP over 1 year (July 2010–July
2011). In particular, we were interested in determining recovery of photosynthesis from
acute effects of oiling versus potential chronic depression of photosynthesis lasting longer
than a few months after oiling. To compare the responses obtained from plants at the high
energy, exposed, and eroding coastline at MP, we also measured plant photosynthesis
at the low energy, protected, and depositional coastline in the lagoon at HI after 1 year
(August 2011). At MP we sampled three treatment areas about 300 m\(^2\) each (Figure 7.3):
one treatment was not impacted by crude oil (control), the second treatment experienced
medium impact (some oil observed on the sediment and plants), and the third treatment
was heavily impacted (plants covered by crude oil extensively) in July 2010. It is impor-
tant to note that the spatial extent of the heavy impact area was not larger than about 5 m
wide \( \times 10 \) m long (50 m\(^2\)). At HI we sampled only at the control treatment and the heavily
oiled treatment (Figure 7.3), there was no medium-oiled treatment. Within each treatment
at both locations, we randomly sampled up to 10 individual plants, taking measurements
on one leaf per plant (see below), at each visit.
Photosynthesis was measured using two instruments. First, chlorophyll fluorescence was measured with a pulsed amplitude modulation (PAM) fluorometer, the Walz Mini-PAM (www.walz.com) to obtain plant stress ($F_v/F_m$) and electron transport rate (ETR). Second, CO$_2$ flux and chlorophyll fluorescence were measured with a LI-6400XT portable photosynthesis and fluorescence system (www.LICOR.com/env) to obtain carbon fixation rates ($\mu$mol CO$_2$/m$^2$/s) and plant stress ($F_v/F_m$). The PAM is able to measure fluorescence very quickly and all measurements were obtained in a few hours during the morning, whereas the LI-COR relies on slower gas-exchange processes requiring repeated visits over the course of 3 days to obtain the same number of replicate measurements. For this reason, the results between instruments were not compared directly.

**Pulsed Amplitude Modulation Chlorophyll Fluorescence**

We used the PAM to measure the chlorophyll fluorescence yield on 10 randomly selected individuals of smooth cordgrass in each of the three treatments at MP during each time point. Plants were not tagged, so each visit likely resulted in us selecting different individuals. Measurements were made at MP on July 21, 2010 (13 days after oiling); October 4, 2010 (88 days); January 19, 2011 (195 days); March 10, 2011 (245 days); and July 7, 2011 (351 days). In addition, we had already made chlorophyll fluorescence yield measurements on smooth cordgrass plants on May 9, 2010 in the lower Pascagoula River delta 18 km away from the east in anticipation of future oil spill impacts, so this data were included in our analysis as a before-impact sample for the control treatment.

The yield measurement was always taken from the middle portion of the second leaf from the top of the plant, leaves used for PAM measurements were always green. To obtain the measurements, the instrument-supplied leaf clip (DLC-8) was attached to the midpoint of the leaf and immediately the chlorophyll fluorescence under ambient sunlight (effective quantum yield [EQY] ) was recorded. Subsequently, the shutter on the leaf clip was closed for a minimum of 15 minutes and the dark-adapted measurement (potential quantum yield [PQY]) was then taken from the same leaf. As there were 10 leaf clips, all 10 individuals were measured within a few minutes of each other.

In addition to these yield measurements, three additional individuals were randomly selected and a rapid light curve (RLC) was measured using the same leaf selection criteria outlined for the yield measurements. The RLC was obtained by measuring the yield over nine incremental light levels (0, 55, 81, 122, 183, 262, 367, 616, and 1115 $\mu$mol photons/m$^2$/s) generated by the halogen lamp inside the PAM. From these measurements the instrument calculates the ETR by the following equation:

$$ETR = EQY \times PAR \times AF \times 0.5$$

where EQY, effective quantum yield; PAR, light level generated by halogen lamp inside the PAM; AF, absorption factor set to instrument default setting of 0.84; and 0.5, 50% of incident light absorbed at photosystem II (PSII) (Walz 1999). The RLC so obtained can be considered as the equivalent of a photosynthesis–irradiance (P–I) curve for the light reactions of photosynthesis. The PAM measurements were typically conducted between 0900 and 1400 hours (CST), with the exact time for each measurement recorded in the instrument memory. Measurements were conducted on sunny days when possible.

In summer 2011, we revisited the HI location and measured chlorophyll fluorescence on plants in the control and the heavily oiled treatments using the same methods outlined above for MP. From this 1-year post-oiling time point we wanted to determine whether the
chronic exposure to residual oil at the HI location was evident as depressed photosynthesis measurements (EQY, PQY, and RLC).

**LI-COR Chlorophyll Fluorescence and Gas Exchange**

We used a LI-6400 XT portable photosynthetic gas exchange and chlorophyll fluorescence system to measure the photosynthesis rate (μmol CO₂/m²/s) and dark-adapted chlorophyll fluorescence (PQY) at each time point. Measurements at MP were conducted monthly between July 2010 and June 2011. No photosynthesis data were able to be collected in December 2010 due to lack of boat availability, nor at the 1-year time point in July 2011 because of a broken instrument that was accidentally exposed to saltwater.

At each visit, we randomly chose up to 15 individuals for measurement of the photosynthesis rate on the middle portion of the second leaf from the top of the plant. Leaves used for LI-COR measurements were not always green, and included a range of healthy to senescing/unhealthy leaves. The photosynthetic rate for each leaf was measured by CO₂ exchange under five photosynthetically active radiation (PAR) intensities: approximately 400, 800, 1200, 1600, and 2000 μmol photons/m²/s. In addition, for each leaf we measured PQY after at least 30-minute dark adaption and used this as an indicator of leaf and individual stress.

To assess the recovery of photosynthesis at the two oiled treatments (medium, heavy), we assumed that their close proximity to the control treatment provided similar environmental conditions. Time series of monthly PQY for each of the three treatments were compared against only the light saturated (1200–2000 μmol photons/m²/s) gas-exchange measurements (P_{max}) from the same individuals, and then the time until the mean response at an impacted treatment was not significantly different from the control response was determined. In a second approach to assess time to recovery, we used all the gas-exchange measurements obtained by the LI-COR to construct the mean P–I curve for each season. Summer was defined as June–September, fall was October–November, winter was December–February, and spring was March–May, based on mean monthly air temperatures. Seasonal mean P–I curves for the heavily oiled and the control treatments were plotted, and recovery determined when the 95% confidence intervals overlapped at all irradiance levels.

**Statistical Analyses**

All plant photosynthesis data were imported from the instruments into Excel (Microsoft Office© v. 2007) and processed prior to analyses, which were performed in JMP Intro v.5 (SAS 2005). The replicate was an individual plant (one leaf measured per plant), with ≤10 plants measured in each treatment (control, oiled) within each location (MP, HI) at each time step (month). Data were tested for normality and homoscedasticity using the Shapiro-Wilk’s and Bartlett’s test, respectively. Data were transformed (log_{10}[Y + 1]), where appropriate, to meet assumptions for subsequent statistical models. The EQY and PQY data (collected with PAM) and F_v/F_m and P_{max} data (collected with LICOR) were each analyzed separately by a two-way mixed model analysis of variance (ANOVA) with treatment (i.e., control vs. medium vs. heavy oil) designated as a fixed effect and month designated as a random effect. Resulting significant treatment × time interaction effects for EQY, PQY (n = 10 plants) and F_v/F_m, P_{max} (n = 2–10 plants) were determined post hoc by separate one-way ANOVA (treatment) tests for each month sampled, with the alpha-level adjusted (Dunn-Sidak) to compensate for multiple pair-wise comparisons (PAM = 5 months,
LI-COR = 10 months). Finally, EQY and PQY data collected with the PAM 1-year postimpact at MP and HI, were tested with a two-way fixed factor ANOVA with treatment (i.e., control vs. oiled) and location considered as fixed effects. For all test results with significantly different means, Tukey’s HSD was used to determine means that were not significantly different. Data for RLC and P–I curves were assessed for differences among means either by comparing the 95% confidence intervals (~2σ) to the curves, or from the slopes and $R^2$ coefficients of the linear regression between control and oiled variables.

#### Results

**Spatial Contamination**

During fall 2010, ORO were found in both sediment and plant tissue samples, but not in animal muscle (both fish and shrimp) samples. Not all locations yielded sufficient animal tissues for analysis (minimum 3 g required), hence the low number of results for these samples (Table 7.1). Only at MP were all three species sampled found to co-occur at the time of collection (Table 7.1). No ORO were detected in sediments or plant tissues from control treatments at GBNERR and MP, both relatively energetic shorelines, or from SB, a more protected shoreline but with strong tidal currents. In contrast, in the low energy lagoon on HI, ORO were detected at the control treatment in both sediment and plant samples despite being almost 300 m away from the oiled treatment, indicating chemical contamination had spread throughout the enclosed lagoon.

ORO were detected in all oiled treatments, but not always in both sediment and plant fractions. Detected concentrations ranged from 101 to 67,500 ppm in the sediments and from 465 to 31,300 ppm in the smooth cordgrass tissues (Table 7.1). There tended to be as much variability among quadrats within the impacted treatment as there was between oiled locations, for example, MP sediments in October 2010 ranged between 0, 6,470, and 67,500 ppm in the three quadrats each located 5 m apart (Table 7.1). This was confirmed by visual observation at the time of collection, with heavy oil coating the sediment surface to a depth of up to 3 cm in the quadrat with the heaviest ORO concentration, whereas no visible oil was seen in the quadrat that gave a result of 0 ppm. This pattern was consistently encountered in oiled treatments that had visible oil at the time of collection (MP, HI, SB), and indicates the degree of patchiness in both the initial oiling event(s) and the subsequent breakdown or removal of oil contaminants by natural processes.

PAHs were generally not detected in samples analyzed from fall 2010. The only PAH detected was chrysene, found in one plant tissue sample each at the oiled treatment from SB (0.47 ppm) and HI (0.68 ppm). As chrysene was found in the absence of other by-products of combustion, such as fluoranthene, pyrene, or benzo[a]anthracene, this result suggests crude oil contamination was subsequently taken up or sorbed into the plant tissues. This conclusion is supported by the dominance of chrysene in the weathered oil collected at MP (see Section Time-Series Contamination).

**Time-Series Contamination**

The oil on the plants at MP in July 2010 (Figure 7.4) was analyzed further to determine the hydrocarbon composition, and compared to a reference oil sample obtained from the
Oil Contamination in Mississippi Salt Marsh Habitats

MC252 well. The Pr/Ph ratio of the MP oil (1.0) is close to that of the MC252 well (0.9), suggesting that MP oil originated from the BP oil spill. The overall hydrocarbon compositions of the MP oil, including alkanes, PAHs, and alkylated PAHs, are similar to the oil collected on the sea surface of northern GOM during the oil spill, a further indication of MC252 origin (Liu et al. 2012). The MC252 oil shows a predominance of \( n \)-alkanes with short carbon chains (C\(_{9-18}\)) at concentrations exceeding 3 mg/mL (\(-3000\) ppm) and a lower concentration of longer carbon chains (C\(_{20-38}\)) present (Figure 7.5a). In comparison, the oil

### TABLE 7.1

Oil Contamination in Each of Three 1-m\(^2\) quadrats (Q1–Q3) Sampled in Fall 2010, 3 Months After Initial Oiling, from Four Locations along the Mississippi Coastline.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Oiling</th>
<th>Q1 MRL</th>
<th>Q2 MRL</th>
<th>Q3 MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment</td>
<td>GBNERR</td>
<td>Control</td>
<td>ND 50</td>
<td>ND 50</td>
<td>ND 50</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>ND 50</td>
<td>ND 50</td>
<td>ND 50</td>
<td>ND 50</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>6,740</td>
<td>2,500</td>
<td>ND 50</td>
<td>67,500</td>
</tr>
<tr>
<td></td>
<td>Horn Island</td>
<td>Control</td>
<td>101</td>
<td>50</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>173</td>
<td>100</td>
<td>109</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>Waveland</td>
<td>Control</td>
<td>ND 50</td>
<td>ND 50</td>
<td>ND 50</td>
</tr>
<tr>
<td></td>
<td>Heavy</td>
<td>3,950</td>
<td>1,500</td>
<td>ND 50</td>
<td>500</td>
</tr>
</tbody>
</table>

| Plants   | GBNERR   | Control  | ND 930 | ND 735 | ND 1,280|
|          | Heavy    | 1,070    | 600    | 1,340  | 963    | 690    |
|          | Heavy    | 150      | 192    | 150    | 1,500  |
|          | Waveland | Control  | ND 1,130 | 31,300 | 13,100 | 9,000 |
|          | Heavy    | 3,360    | 1,020  | 31,300 | 13,100 | 9,000  |

| Animals  | GBNERR   | Control  | ND 268 |
|          | Heavy    |          |
|          | Marsh Point | Control | ND 485 | ND 517 | ND 600 |
|          | Heavy    | ND 500   |
|          | Horn Island | Control | ND 526 |
|          | Heavy    | ND 441   | ND 455 |
|          | Waveland | Control  | ND 455 |
|          | Heavy    |          |

**Note:** Grand Bay National Estuarine Research Reserve (GBNERR) and Marsh Point are both eroding shorelines, whereas Horn Island Garden Pond and Waveland are both protected, depositional shorelines. Sediments, plant tissues, and animal tissues (\( P = Palaeomonetes \) spp., Fs = Fundulus similis, Fg = Fundulus grandis) were analyzed for the concentration (mg/kg) of oil range organics (C\(_{19-36}\)) using EPA method 8015C. MRL is minimum reporting limit that varies depending on the sample dilution, ND indicates ORO was below detection for that sample.

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FIGURE 7.4
(See color insert.) Time-series photos of oil contamination and smooth cordgrass plant responses at Marsh Point from July 2010 (2–13 days after oiling) until July 2011 (351 days after oiling). The left panels show the same general area of the heavily oiled treatment, marked by either two tall bamboo marker posts or a short, red-painted wooden stake. The right panels show close-ups of the 1-m² quadrats that were sampled for contamination and show the condition of the weathered oil over time, as well as the recovery of the initially heavily oiled plants through the seasons.
from plants at MP was dominated by intermediate \( n \)-alkanes (\( C_{19} - C_{26} \) at a concentration \( \geq 0.6 \) mg/g TSEM), and longer carbon chains (\( C_{27} - C_{38} \) at a concentration \( \leq 0.6 \) mg/g TSEM) (Figure 7.5b). There was a very low concentration of short carbon chains (\( C_{10} - C_{18} \)), unlike what was observed in the reference MC252 oil sample.

Naphthalene dominates PAHs in the MC252 oil, accounting for 64% of the total PAHs, followed by phenanthrene and fluorene (Figure 7.5c). Chrysene and other PAHs are minor components. These distribution patterns of alkanes and PAHs are typical of fresh crude oil samples. For the PAHs in the MP oil, naphthalene accounted for only 9% of the total, and other PAHs with 2–3 aromatic rings, including fluorene and phenanthrene, were minor components relative to the reference MC252 oil (Figure 7.5d). Chrysene became the dominant (53%) PAH in the MP oil. Other PAHs with a high number of aromatic rings (>4), from benzo[b]fluoranthene (BbF) to benzo[ghi]perylene (BgP), became more concentrated relative to the reference oil (Figure 7.5). These results indicate that the oil present at MP in July 2010 was substantially weathered, as the low-molecular-weight \( n \)-alkanes and PAHs were preferentially lost. This agrees with field observations at the time of a rust-red colored, very sticky, tar-like consistency, oil coating on the plants (Figure 7.4). It is also evident that PAHs overall had been more weathered than the alkanes, as the ratio of total alkanes/PAHs was 70 in the crude oil, but was as high as 1400 in the MP oil. Thus, by the time the oil had reached the salt marsh it had weathered to an extent that it was less toxic to smooth cordgrass relative to the potential toxicity of oil at the well, based on the PAHs.

At MP (the high-energy coastline), the initial sampling in July 2010 (13 days postimpact) was from a single quadrat in each of the control and heavily oiled treatments (Table 7.2). ORO concentrations at this time were orders of magnitude higher in the plant tissues (2,660–137,000 ppm) than in the sediments (32–68 ppm), concurring with observations that the oil had arrived during a storm-driven high tide and coated the plants within 3–5 m of the shoreline. Little oil was deposited on the sediments at the time of initial oiling (Figure 7.4). In later months, the pattern of oiling changed with substantially more ORO found in sediments in October 2010 (88 days postimpact), ranging from 6,740 to 67,500 ppm, compared to the plant tissues where no oil was detected in any of the three quadrats sampled (Table 7.2). This concurs with observations made at this sampling event, which indicated that regrowth of new plant shoots had occurred since the initial oiling and the oil-coated shoots had died, falling over and leaving the sediment prone to oil transfer from the plant detritus (Figure 7.4). During the spring (March 2011), ORO were detected in sediments (96 ppm) and plant tissues of one quadrat in the control treatment, but at substantially lower concentrations than those found in the quadrats (\( \geq 3600 \) ppm) in the heavily oiled treatment (Table 7.2). Finally, 1 year after oiling, samples from July 2011 (Figure 7.4, Table 7.2) indicate minimal sediment contamination (164 ppm), but some residual contamination occurred in plant tissues at both the control treatment (563–909 ppm) and the heavily oiled treatment (1270 ppm). These final results suggested that oil contamination may have spread out from the original impact area and resulted in low-level contamination over a broader area, including the plants at the control treatment.

At HI (the low-energy coastline) sampling occurred only twice, in fall 2010 and 1-year postimpact in summer 2011 (Table 7.2). In November 2010, we detected oil contamination in both sediments and plant tissues at both the control and heavily oiled treatments, with greater ORO concentrations at the oiled treatment than the control (Table 7.2). As the HI location is in a lagoon, we anticipated that oil would persist in the sediments for longer than at the MP location. Results from August 2011 confirm this with persistent low-level contamination in all three quadrats sampled at the oil-impacted treatment (59–86 ppm),
Impact of Oil Spill Disasters on Marine Habitats and Fisheries in North America

FIGURE 7.5
Concentrations of hydrocarbons in crude oil from the Macondo Canyon well (MC252), and weathered oil attached to smooth cordgrass tissues at Marsh Point in July 2010. (Modified from Liu et al., Environ. Res. Lett. 7, e035302, 2012.) The measured hydrocarbons include (a, b) \( n \)-alkanes (\( C_9-C_{38} \)), pristane (Pr), and phytane (Ph), and (c, d) PAHs. The hydrocarbon concentrations in MP oil were normalized to total solvent-extractable materials. These chemical fingerprints indicate substantial weathering of the oil had occurred by the time it washed ashore in the marsh.
whereas no ORO were found in sediments at the control treatment (Table 7.2). Results from plant tissue samples also suggest that ORO contamination 1 year after initial oiling was greater at the oiled treatment (604 ppm) than at the control treatment (362 ppm). Like the situation at MP, these final plant tissue results suggested that oil contamination may have spread out from the original impact area and resulted in low-level contamination over a broader area, including the previously unoiled control treatment within the lagoon.

In addition to the results reported in Table 7.2 from the heavily oiled treatment at HI, one extra sediment and two extra plant tissue samples were collected in August 2011 from an area with visibly oiled dead plant stems that had spent inflorescences on them,

TABLE 7.2
Oil Contamination in Each of Three 1-m² Quadrats (Q1–Q3) Sampled (a) Approximately Quarterly (Only One Quadrat was Sampled in July 2010) from Marsh Point, an Erosional Shoreline and (b) Sampled Semiannually from Horn Island Garden Pond, a Depositional Shoreline

<table>
<thead>
<tr>
<th>Sample</th>
<th>Month</th>
<th>Oiling</th>
<th>Q1</th>
<th>MRL</th>
<th>Q2</th>
<th>MRL</th>
<th>Q3</th>
<th>MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment</td>
<td>July-10</td>
<td>Control</td>
<td>69</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>32</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>October-10</td>
<td>Control</td>
<td>ND</td>
<td>50</td>
<td>ND</td>
<td>50</td>
<td>ND</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>6,740</td>
<td>2,500</td>
<td>ND</td>
<td>50</td>
<td>67,500</td>
<td>2,500</td>
</tr>
<tr>
<td></td>
<td>March-11</td>
<td>Control</td>
<td>ND</td>
<td>50</td>
<td>ND</td>
<td>50</td>
<td>97</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>7,040</td>
<td>500</td>
<td>ND</td>
<td>50</td>
<td>3,600</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>July-11</td>
<td>Control</td>
<td>ND</td>
<td>50</td>
<td>ND</td>
<td>50</td>
<td>ND</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>ND</td>
<td>50</td>
<td>164</td>
<td>50</td>
<td>ND</td>
<td>50</td>
</tr>
<tr>
<td>Plants</td>
<td>July-10</td>
<td>Control</td>
<td>2,660</td>
<td>2,030</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>137,000</td>
<td>54,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>October-10</td>
<td>Control</td>
<td>ND</td>
<td>300</td>
<td>ND</td>
<td>300</td>
<td>ND</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>ND</td>
<td>750</td>
<td>ND</td>
<td>600</td>
<td>ND</td>
<td>1,500</td>
</tr>
<tr>
<td></td>
<td>March-11</td>
<td>Control</td>
<td>ND</td>
<td>600</td>
<td>ND</td>
<td>1,350</td>
<td>953</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>375</td>
<td>300</td>
<td>ND</td>
<td>900</td>
<td>ND</td>
<td>3,000</td>
</tr>
<tr>
<td></td>
<td>July-11</td>
<td>Control</td>
<td>678</td>
<td>660</td>
<td>909</td>
<td>570</td>
<td>563</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>ND</td>
<td>810</td>
<td>ND</td>
<td>705</td>
<td>1,270</td>
<td>705</td>
</tr>
<tr>
<td>Horn Island—Depositional</td>
<td>November-10</td>
<td>Control</td>
<td>101</td>
<td>50</td>
<td>67</td>
<td>50</td>
<td>ND</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>August-11</td>
<td>Control</td>
<td>ND</td>
<td>50</td>
<td>ND</td>
<td>50</td>
<td>ND</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>65</td>
<td>50</td>
<td>87</td>
<td>50</td>
<td>59</td>
<td>50</td>
</tr>
<tr>
<td>Plants</td>
<td>November-10</td>
<td>Control</td>
<td>ND</td>
<td>150</td>
<td>ND</td>
<td>192</td>
<td>150</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>547</td>
<td>360</td>
<td>1,740</td>
<td>1,500</td>
<td>465</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>August-11</td>
<td>Control</td>
<td>362</td>
<td>300</td>
<td>ND</td>
<td>1,020</td>
<td>ND</td>
<td>510</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heavy</td>
<td>ND</td>
<td>1,800</td>
<td>ND</td>
<td>690</td>
<td>604</td>
<td>420</td>
</tr>
</tbody>
</table>

Note: Sediments and plant tissues were analyzed for the concentration (mg/kg) of oil range organics (C₁₉–C₃₆) using EPA method 8015C, MRL is minimum reporting limit that varies depending on the sample dilution, ND indicates ORO was below detection for that sample.
indicating they were standing detritus leftover from the 2010 growing season. The sediment sample collected from immediately adjacent to the dead stems was found to have 1250 ppm (MRL = 100), and the 2010 plant detritus samples had 8430 ppm (MRL = 6480) of ORO, whereas the plant tissues that were green and from new growth in 2011 had ND (MRL = 3300) contamination. These three extra samples indicate that at the heavily oiled treatment 1 year later, there was still visible oil contamination on the previous year’s plant detritus and contamination in the adjacent sediments, but that new plant growth in the same sediments was not detectably contaminated. This result highlights the very patchy nature of the oil impacts and the difficulty in determining oil impacts to plant function based on contamination data alone.

**Photosynthesis Responses to Oil Contamination**

*Pulsed Amplitude Modulation Chlorophyll Fluorescence at Marsh Point*

Optimal values of PQY range from 0.75 to 0.83 in field measurements (Krause and Weis 1991; Maxwell and Johnson 2000), with EQY often slightly depressed compared to PQY due to photochemical quenching (i.e., active electron transport). Both the EQY and PQY of the plants at the control treatment at MP remained above 0.6, even during the winter when this species underwent senescence (Figure 7.6). In contrast, both EQY ($F_{2,27} = 179, p < .0001$) and PQY ($F_{2,27} = 80.9, p < .0001$) were significantly depressed immediately (13 days, July 2010) after the oil impact compared to the control plants, and yields in the heavily oiled plants were significantly lower than those in the medium impact treatment. Quantum yields show rapid recovery of photosynthesis in plants at the medium and heavily oiled treatments to values not significantly different from plants at the control treatment by 88 days (October 2010) post-spill (Figure 7.6). Quantum yields remained depressed in plants at all three treatments during the winter months (88–195 days), but with no significant difference among treatments. The yields recovered to preimpact values by March 2011 (day 245), however, PQY was significantly lower ($F_{2,27} = 11.7, p < .0002$) in plants from the two oil-impacted treatments than plants at the control treatment. In July 2011 (351 days), there was no longer any indication of chronic stress in plants at the two oiled treatments compared to the control at MP, suggesting that a complete recovery of photosynthesis had occurred (Figure 7.6).

The RLC results support this same trajectory of rapid recovery when comparing plants from the heavily oiled treatment to plants from the control treatment at MP (Figure 7.7a). Data obtained from the medium-impacted plants are not plotted, but were aligned between the responses shown for the control and the heavily oiled plants. The P–I curves on day 13 show that plants at the heavily oiled treatment have a very depressed response (ETR < 5 μmol electrons/m²/s) compared to the plants at the control treatment (ETR > 10 μmol electrons/m²/s). In all subsequent sampling dates (days 88–351), the P–I curves for plants at the two treatments are not substantially different (Figure 7.7a). When we plotted the data in control versus impact space and fit a straight line with intercept of zero, the slope of the fit for days 88–351 ranged from 0.89 ($R^2 = 0.957$) to 1.17 ($R^2 = 0.992$), with a slope of 1.0 being a perfect match. Also, 95% confidence intervals for the means included the 1:1 line indicating no significant difference between the ETR obtained from the heavily impacted plants compared to the controls. However, immediately after the initial oiling in July 2010, the slope of the fit was 0.18 ($R^2 = 0.187$) and was substantially less than on the subsequent days. The 95% confidence intervals also did not overlap the 1:1 line, indicating a significant depression of the ETR in plants at the heavily oiled treatment compared to the control at MP.
Oil Contamination in Mississippi Salt Marsh Habitats

The chlorophyll fluorescence data obtained with the PAM indicate a rapid recovery of the light reactions of photosynthesis in the plants at both the medium and the heavily oiled treatments to levels observed in plants at the control treatment by October 2010, 88 days after the impact (Figures 7.6 and 7.7). The results obtained during winter months tended to be depressed compared to spring and summer months, reflecting winter senescence known to occur in this species. The rapid recovery of photosynthesis, as measured by chlorophyll fluorescence, indicates a short acute impact and minimal chronic impact of the oil to smooth cordgrass at MP.

LI-COR Fluorescence and Gas Exchange at Marsh Point

Chlorophyll fluorescence (PQY) measurements collected with the LI-COR at MP showed a recovery of photosynthesis during the fall 2010 months to control levels (Figure 7.8), whereas CO₂ flux (Pₘₐₓ) remained depressed in the control and medium impact treatments. Statistical analyses of the recovery were complicated by the lack of control data for August, September, and December 2010, so these months had to be dropped from the dataset. The
PQY results showed a significant ($F_{2,27} = 122, p < .0001$) difference in July 2010 among the three treatments at MP. Plants at the medium impact treatment were more stressed than the control plants and those at the heavily oiled treatment were more stressed than the

**FIGURE 7.7**

(a) Rapid light curves (RLC) of photosynthesis measured on smooth cordgrass in two treatments (control and heavily oiled) at Marsh Point over 1 year showing the rapid recovery of the light reaction of photosynthesis in oil-impacted plants (within 88 days) to performance levels similar to the adjacent control plants. Legend: C is the control, H is the heavily oiled treatment, and the number is days postimpact. For example, H13 = heavily oiled treatment 13 days after impact and C351 = control treatment at 351 days (1 year) after impact. (b) Scatterplot of observed (control treatment) versus predicted (heavily oiled) for the RLC data. Linear regression ($y = mx + b$) and $R^2$ values for each date are presented, 95% confidence intervals are not shown to improve clarity.

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Oil Contamination in Mississippi Salt Marsh Habitats

Medium-impacted plants (Figure 7.8). Monthly measurements from August 2010 until June 2011 at MP showed a very rapid recovery of PQY to control values (≥ 0.70) within 60–90 days (Figure 7.8a). During October 2010, only the heavily oiled plants had a mean PQY significantly lower ($F_{1,18} = 15.4, p < .001$) than that measured in the control plants. PQY declined in all three treatments in November and remained depressed below 0.7 until March 2011. Smooth cordgrass has a seasonal dieback in winter resulting in depressed photosynthesis compared to summer (Figure 7.8). From March until June 2011, when measurements stopped, PQY remained high (>0.75) and steady at all three treatments. The chlorophyll fluorescence data indicate there was no chronic stress from the 2010 oil impacts in the plants measured at all three treatments in June 2011 at MP.

During July 2010, $P_{\text{max}}$ measurements taken at saturating irradiances (1200–2000 μmol photons/m²/s) also showed a significant ($F_{2,27} = 114, p < .0001$) difference among the three treatments, with control > medium > heavily oiled plants (Figure 7.8b). During the late summer and fall of 2010, the plants at the control and medium impact treatment exhibited a decline in $P_{\text{max}}$, whereas plants in the heavily oiled treatment initially showed an increase in $P_{\text{max}}$ in August and September, before declining again in October–November. By October 2010, the heavily oiled plants had a mean $P_{\text{max}}$ significantly lower ($F_{1,18} = 23.3, p < .0001$) than that measured in the control plants, whereas in November 2010, the heavily oiled plants were significantly lower ($F_{2,27} = 9.1, p < .0009$) than both the medium impact...
and control plants (Figure 7.8b). By November 2010, $P_{\text{max}}$ declined at all three treatments and then remained below 20 μmol CO$_2$/m$^2$/s during the winter. In March 2011, $P_{\text{max}}$ began to increase with the spring regrowth and returned to a range of 20–30 μmol CO$_2$/m$^2$/s (Figure 7.8b). The $P_{\text{max}}$ measurements also indicate there was no chronic stress from the 2010 oil impacts in the plants measured at all three treatments in June 2011 at MP. The LI-COR data confirm our hypothesis that chlorophyll fluorescence recovers very rapidly from acute stress (within less than 2 months after the impact), whereas the gas-exchange measurements show a slower recovery with some chronic depression evident in the heavily oiled plants for up to 4 months after the impact.

Seasonal mean P–I curves measured in smooth cordgrass at the control and heavily oiled treatments at MP also indicated that recovery of the heavily oiled plants took between 4 and 6 months (Figure 7.9). Initially in summer 2010, immediately following oiling of the plants, the P–I curve for the heavily oiled plants was essentially flat near zero, whereas the P–I curve of the control plants had a $P_{\text{max}}$ around 45 μmol CO$_2$/m$^2$/s (Figure 7.9). Over the course of the following year, the P–I curve of the heavily oiled plants recovered back to levels measured in the same season in the adjacent control plants, with convergence of the P–I curves by winter (December–February). The depression of the P–I curve in control plants during the autumn ($P_{\text{max}} = 20$) and winter ($P_{\text{max}} = 10$) compared to spring ($P_{\text{max}} = 25$) and summer ($P_{\text{max}} = 45$ μmol CO$_2$/m$^2$/s) is an inherent natural response of this species to cold air temperatures and reduced day-lengths. The P–I curve data further strengthens...
the previous findings that plants in the heavily oiled MP treatment required until winter/spring to fully recover photosynthesis to rates seen in the control plants, with no evidence of chronic effects by 1 year (July 2011) after the oil impacts.

One-Year Assessment

In July 2011, the quantum yields of plants in the control treatment and the heavily oiled treatment at the two locations, MP and HI, were compared. There were significant differences due to location for both EQY ($F_{1,35} = 9.05, p < .005$) and PQY ($F_{1,35} = 22.1, p < .0001$), and significant interaction effects (location $\times$ treatment) for EQY ($F_{1,35} = 7.91, p < .008$) and PQY ($F_{1,35} = 8.37, p < .007$). At the high-energy MP location, the quantum yields were not significantly different between the plants at the control and heavily oiled treatments (Figure 7.10). In contrast, at the low energy HI location, the mean EQY ($t_{1,18} = 2.47, p < .011$) and PQY ($t_{1,18} = 1.84, p < .041$) were both significantly lower in the plants at the heavily oiled treatment compared to the plants at the control treatment (Figure 7.10). Variances were also much greater in the heavily oiled treatments at both MP and HI compared to the control, reflecting a range of plants from those that were very stressed (EQY $< 0.55$) to those that exhibited less stress (PQY $> 0.8$).

The RLCs support this finding with the 95% confidence intervals of the mean ETR overlapping at all irradiances for the control and heavily oiled treatments in the MP location (Figure 7.11a). The slope of the linear fit between control and heavily oiled data points was 1.034 with an $R^2$ of 0.99. As expected from the previous quantum yield results, at HI the RLC confidence intervals did not overlap for the majority of the curve, exceptions were 55 and 367 μmol photons/m²/s (Figure 7.11b). The slope of the linear fit between control and heavily oiled data points was 0.788 with an $R^2$ of 0.86. These results indicate that the plants at the heavily oiled treatment continue to exhibit chronic depression of photosynthesis, as measured by chlorophyll fluorescence, up to 1 year after the initial oil impacts.

Plant stress in the heavily oiled treatment at MP was less than at HI as indicated by the quantum yields and RLC results (Figure 7.11). Combining these data with the previous results from the contaminant analyses (Table 7.2), the summer 2011 findings suggest that there were major differences in recovery after 1 year between MP and HI. At the depositional location (HI) there was prolonged and ongoing exposure to oil residues in the sediments that possibly continue to cause a chronic depression of photosynthesis in the smooth cordgrass plants. In comparison, at the erosional location (MP), there were fewer oil residues in the sediments, and the plants appeared to have recovered from the oiling impacts.

Discussion

Contamination

The DWH disaster was the largest accidental marine oil spill in U.S. history and released about 4.9 million barrels ($7.0 \times 10^6 \text{ m}^3$) of crude oil into the open ocean (Crone and Tolstoy 2010). Oil gradually dispersed through the water column as its buoyancy caused it to rise toward the surface of the ocean from the 1544 m deep source at the MC252 wellhead (Camilli et al. 2010; McNutt et al. 2011; Oil Spill Commission 2011). Oil in the water was
visible from April 24, 2010 onward (Oil Spill Commission 2011) throughout much of the summer in 2010 and was extensively monitored and mapped using satellite and aerial imagery (U.S. Government 2010; White House 2010; McNutt et al. 2011). Despite the leaking wellhead being contained by July 15, 2010, oil from the extensive offshore slicks that cumulatively covered up to 180,000 km$^2$ continued to disperse and eventually contaminated more than 1050 km of shoreline (Skytruth 2010). Oil periodically came ashore in Mississippi salt marshes, with peak frequency of oiling from early June until late September 2010. Timing of heavy oiling in the marsh was observed to coincide with tropical storm systems, including Hurricane Alex (June 30–July 1), Tropical Storm Bonnie (July 24–25), and Tropical Depression number 5 (August 12–13), which all brought strong southerly winds.

FIGURE 7.10
Boxplots comparing (a) Effective quantum yield (EQY) and (b) Potential quantum yield (PQY) responses at Marsh Point ([MP] erosional, exposed shoreline) versus Horn Island ([HI] depositional, protected shoreline) in July 2011, 1 year after initial oil impacts. Significant effects due to treatment and location are indicated by the different letter groups.
Oil Contamination in Mississippi Salt Marsh Habitats

A storm surge that pushed ashore water contaminated with oil (NOAA 2010). The elevated tides, measured at the Gulf Coast Research Laboratory pier, associated with the storm surge (+0.6 m above MHHW) contributed to further inland penetration of the floating oil slicks than would otherwise have occurred with normal tidal amplitudes (0.47 m). Despite this, most oil contamination was primarily to smooth cordgrass within <10 m from open water in most areas, affecting the coastal fringe disproportionately.

The first part of our study assessed crude oil impacts in sediments, plant tissues, and selected nekton in salt marsh habitats along the Mississippi coastline. This information will be invaluable in determining the long-term ecological impacts and potential food web implications of the oil, which ended up being trapped in the delicate coastal marsh habitats of Mississippi and the northern GOM. Our contaminant sampling began after funds were obtained, and occurred about 90 days after the wellhead had been effectively sealed. This allowed sufficient time for any oil remaining in the water column to make it to the

FIGURE 7.11
Rapid light curves (RLC) of photosynthesis measured on smooth cordgrass at two treatments (control and heavily oiled) within (a) Marsh Point (erosional, exposed shoreline) and (b) Horn Island (depositional, protected shoreline) locations in July 2011, 1 year after initial oil impacts. 95% Confidence intervals of the mean are plotted as dashed lines.
Impacts of Oil Spill Disasters on Marine Habitats and Fisheries in North America

shoreline and begin to breakdown into less harmful constituents at the time of our synoptic coast-wide contaminant sampling in fall 2010. The areas we chose to study in detail were locations with prior documentation (photos, GPS coordinates) of oil impacts throughout summer 2010. The four areas selected for our study encompass marsh areas along the Mississippi mainland and barrier island coastlines and were among the most heavily contaminated locations. Despite this, the total area of heavily impacted marsh remained less than a few hundred square meters, and within this area, oil was spatially highly variable even at the sub-meter scale. Some of the heavily oiled areas were $<5 \times 10^2$ m$^2$ in total, and substantial patchiness of the oil within this size area was also observed. Many of the oil patches that were washed ashore were much smaller than this, on the order of $<0.25$ m$^2$, making it hard to detect without significant sampling effort on a fine spatial scale (1 m$^2$ or finer). Because of this highly localized impact, it was important to use a hierarchical sampling approach, based on prior knowledge of impact locations, to capture both within treatment (replicate quadrats) and among treatment (control vs. oil impacted) differences within a single location. Much of the shoreline remained clear of heavy oiling, making it difficult to extrapolate our findings to a Gulf-wide scale of contamination.

Our results suggest that the MC252 oil that reached Mississippi’s salt marshes was highly weathered, which lessened the toxic impacts that potentially could have occurred with a more proximate spill location. In general, PAHs were not detected in the sediment or plant samples with the exception of two samples that contained low ($<1$ ppm) concentrations of chrysene. Sediment sampling by the U.S. EPA (EPA 2010) also found chrysene and other PAH contaminants at very low concentrations (2.9–39 ppb) in beach sediments off Bay St. Louis (report number BCH01-SD-201008) and Pascagoula (report number BCH04-SD-201008) in late summer 2010, whereas no PAH contamination was reported from a subtidal sediment sample near Ocean Springs (report number R4-10-B-SD-09152010). Earlier sampling prior to oil impacts at these locations in May 2010 found no PAH contaminants in the sediments (EPA 2010). These findings are further supported by the extensive “fingerprinting” of a single sample of weathered oil from MP immediately following the initial oiling in July 2010, where chrysene was the dominant PAH present in the sample. As PAHs are the more toxic fraction compared to long-chain alkanes, the lack of substantial PAH contamination in the samples from fall 2010 is a positive finding, indicating a less toxic form of weathered oil contamination was found in the salt marsh. The contamination detected in patches where oil was generally still visible at the time of sampling was composed of mainly longer carbon-chain $n$-alkanes ($C_{19}$–$C_{38}$), which have lower acute toxicity (Lin et al. 2012). ORO were always detected analytically when oil was visible at the time of sampling, but were also found in areas where oil was no longer visible (e.g., GBNERR, HI). When oil was not readily visible at the time of sample collection, the $n$-alkanes were mainly found in plant tissues. Without further petroleum biomarker analysis, it is difficult to evaluate the degree of contamination, as $n$-alkanes are also sourced from plant tissues such as cuticles. Some contamination of plant samples was found in the adjacent control treatment in summer 2011 at both MP and HI, suggesting that oil may have become dispersed along the coastline from the original impact site and now affected a wider area, albeit at a much reduced concentration.

No oil was found in any of the animal muscle tissue samples analyzed, suggesting no or only limited trophic transfer had occurred. However, the limited number of samples that satisfied the requirement for the minimum amount of sample needed (3 g) to detect contamination affects the robustness of this conclusion. An extensive and better designed sampling strategy is required to determine whether habitat contamination might be affecting the lower trophic-level populations in salt marshes (Kahn et al. 2005; Roth and
Baltz 2009; Whitehead et al. 2011). Some scientific studies conducted on offshore planktonic and larval populations detected potential contamination of the lower trophic guilds with entrained oil (Graham et al. 2010; Chanton et al. 2012; Ortmann et al. 2012; Vestheim et al. 2012), however, other studies indicated nondetectable contamination of commercial seafood or no discernible population response at higher trophic levels (Fodrie and Heck 2011; NOAA 2011; Carmichael et al. 2012). At the time of publication, no studies have conclusively linked oil-contaminated plant material as a source of ingested pollutants in coastal marine biota along the northern GOM.

Location-Specific Oil Impacts

The second part of our study assessed plant responses to crude oil impacts in the sediments and on plant tissues at two salt marsh locations with contrasting coastlines: a high energy, exposed, and eroding coastline at MP and a low energy, protected, and depositional coastline in a lagoon at HI. Salt marshes are highly sensitive to oil contamination (Gundlach and Hayes 1978; Jensen et al. 1998), and are ranked by NOAA Office of Response and Restoration as 10 out of 10 in terms of vulnerability on their Environmental Sensitivity Index (NOAA 2012). For this reason, cleanup crews that were effective at removing oil that washed ashore on beaches were not able to remove oil in the salt marshes effectively, and in many instances were tasked not to do so in an effort to minimize further damage (Shogren and Gonyea 2010).

During the DWH oil spill and after the well was closed, more than 1050 km of shorelines along the GOM were exposed to weathered crude oil, with 210 km of those designated as moderately to heavily oiled, and 75 km of these occurred in Louisiana (Oil Spill Commission 2011; Silliman et al. 2012). Previous studies on oil spill impacts to GOM marsh plants have focused on Louisiana because spill events occur more frequently there, and there are larger marsh areas (Pezeshki and DeLaune 1993; Hester and Mendelssohn 2000; Pezeshki et al. 2000; Ko and Day 2004; Mendelssohn et al. 2012; Mishra et al. 2012). Some studies concerning the impact of the DWH oil spill on vegetation have been published recently. Mishra et al. (2012) assessed the ecological impact on the salt marshes along the southeastern Louisiana coast using photosynthetic capacity and physiological status through satellite and ground truth data, and found extensive reduction in photosynthetic activity during the peak of the growing season in 2010. Lin and Mendelssohn (2012) documented variable impacts depending on oiling intensity in the Bay Jimmy area of northern Barataria Bay, Louisiana. As of the fall of 2011, many of the most heavily oiled shorelines at that location had minimal to no recovery (Mendelssohn et al. 2012). In Mississippi, some salt marshes experienced crude oil impacts in summer 2010 and our four study locations represent the high-end of oil contamination for the documented impact locations in the state. Despite these and other ongoing studies, data on the impact and recovery of the GOM plants are still sparse. Nevertheless, this chapter enhances our understanding of the impacts of the DWH oil spill on photosynthesis of smooth cordgrass, the dominant marsh plant in this ecosystem.

As most of the time-series research documenting acute and chronic impacts to photosynthesis was done at the MP location, we will discuss these results first. In July 2010, almost 2 weeks after the initial arrival of the oil during Tropical Depression number 2 on July 8–9 (NOAA 2010), we were able to obtain initial data on oil contamination and plant stress using the PAM and LI-COR. The thick oil coating immediately affected leaves within the heavily oiled treatment and appears to have resulted in acute leaf effects by blocking transpiration and gas exchange through the stomata (Ferrell et al. 1984; Snedaker et al.
This oiling also increased leaf temperatures, which caused them to absorb large quantities of infrared solar radiation and resulted in leaf mortality during the first month. Temperature over the heavily oiled plants was discernibly higher than at the adjacent control treatment when collecting the chlorophyll fluorescence data during the midday hours of an already hot summer day. At the beginning of the crude oil impact (July 2010), the concentration of ORO was high on the plants and low in the sediment. Despite the acute oiling stress and loss of existing leaves, the plants at MP were able to recover by growing new leaves and shoots from intact, unoiled roots within 2–3 months after the initial contamination. The oil concentration on the plants decreased over time but increased on the sediment because of gravitational migration of the oil down to the sediment surface. New growth was evident in both the medium and heavily oiled treatments by the October 2010 sampling event, 88 days after initial oiling, with the oiled dead leaves and stems laying prone on the sediment surface (Figure 7.4). This rapid regrowth of new leaves and shoots from the intact root–rhizome complex afforded the smooth cordgrass plants a short window of opportunity for additional carbon fixation and energy storage prior to the onset of winter senescence, and contributed to a successful recovery the following spring. It is likely that an oiling event later in the year may have resulted in higher mortalities of individuals, as the plants would not have been able to store sufficient reserves to survive dormancy for a prolonged period of many months (Baker 1971; Webb et al. 1985).

A second potential reason for the quick natural degradation of the remaining oil residues on the plants and on the sediments at the MP location over the course of the year-long study is the physical break down of the tar-mats and their removal by waves and tides. Natural degradation processes of the oil in the marsh not only include weathering (temperature, sunlight) but also physical removal by waves and tides (Mendelssohn et al. 2012). In particular, locations such as MP and GBNERR, which are exposed to the predominant southeasterly winds that are common during summer, experienced substantial tidal “cleaning.” Oil was removed during high tides and during periods of strong wave action created by tropical storms in the GOM. Warm temperatures, often exceeding 35°C during the day, are also common in the region from June through September promoting rapid volatilization of lighter carbon fractions. Oil that was observed to persist over time at MP quickly became more asphalt-like in its appearance and consistency, which correlates to reduced toxicity (Irwin et al. 1997). At least one location that was sampled is on a well-documented eroding shoreline, Point Aux Chenes in GBNERR, having lost 2.5 m of coastline per year, on average (Hilbert 2006; Otvos and Carter 2008). This means that the oil-impacted sediments and plants could be lost to open coastal waters within 1–3 years. Similar erosion rates have occurred in some Louisiana marshes (Day et al. 2000; Silliman et al. 2012), suggesting similar fates to oiled salt marsh habitats in the Mississippi River Delta region.

In contrast to the rapid removal of oil from sediments at MP, the sediment inside the protected lagoon at HI was still contaminated (59–87 ppm) by oil in August 2011. At this location, plants exhibited significantly reduced photosynthesis compared to the HI control treatment and previously oiled plants at MP. The difference in residence times between the erosional edge location (MP) and lagoon location (HI), in part, helps to explain why oil persisted in HI sediments. Oiled and standing dead shoots from 2010 were also still present at HI, and sampling of these confirmed there was still heavy oil contamination (ORO = 8340 ppm), further indicating the lack of removal of the contaminants from this low-energy environment. A location with similar characteristics to HI is Bay Jimmy in northern Barataria Bay, Louisiana, that is also a low-energy shoreline with chronic DWH oil pollution problems (Schleifstein 2012; Silliman et al. 2012). Despite cleanup efforts,
oil persists over 1 year later and is causing ongoing plant mortality and loss of coastline (Silliman et al. 2012). Oil trapped in waterlogged fine sediments is known to pose a long-term threat (Teal et al. 1992; Burns et al. 1994) in low-energy coastlines, explaining why salt marshes and mangroves rank as the most susceptible coastal habitats to oil spills (Michel et al. 1978; Jensen et al. 1998). This is because the anaerobic conditions in these sediments inhibit the complete breakdown of oil, resulting in pools of organic and heavy metal pollutants that are extremely slow to degrade (Lin et al. 1999; Andrade et al. 2004). The eventual result is plant mortality from root die-off (Proffitt et al. 1997; Ko and Day 2004), and salt marsh recovery can be further inhibited by new recruits that also fail to establish in the contaminated sediments. In both salt marsh and mangrove habitats, this is the key reason that oil pollution poses a threat of chronic stress in low-energy locations (Proffitt et al. 1997; Snedaker et al. 2001).

These examples from Mississippi and Louisiana demonstrate the importance of coastal energetics in the persistence of oil pollutants and their chronic toxicity to the vegetation. Salt marshes in the GOM are often found along eroding edges and are constantly migrating shoreward in response to coastline erosion (DeLaune et al. 1983; Turner 1990). In sections of coastline that are more exposed and are eroding, oil was removed rapidly by tidal and wave energy and the plants are recovering with new growth. If the plant roots are killed by oil contamination, however, the resulting erosion of the sediments will convert the previously oiled coastal fringe into open-water subtidal habitat (Silliman et al. 2012), diluting and dispersing the remaining hydrocarbons back into the water column.

**Acute and Chronic Photosynthesis Responses**

Chlorophyll fluorescence is a well-established technique to rapidly and noninvasively measure photophysiological processes in vivo and has been used successfully to demonstrate physiological stress in a wide variety of plant species (Critchley and Smilie 1981; Havaux and Lannoye 1983; Bowyer et al. 1991; Filiault and Stier 1999; Maxwell and Johnson 2000). Chlorophyll fluorescence of PSII can provide an instantaneous measure of the EQY under prevailing ambient light conditions (Genty et al. 1989). Alternatively, more standardized differences among leaves can also be determined by measuring the PQY of dark-adapted samples. Generally, the maximum possible proportion of the solar energy absorbed into photosynthesis is around 83%, equivalent to a quantum yield of 0.830 (Maxwell and Johnson 2000). As plants become stressed, reductions in the quantum yield (either EQY or PQY) indicate a reduction in the efficiency with which light is converted to photosynthetic product and subsequently plant growth or reproductive output (Schulze and Caldwell 1990; Krause and Weis 1991; Rohacek and Bartak 1999).

Another technique commonly used to quantify photosynthesis is by measuring leaf gas exchange (Šesták et al. 1971; Farquhar et al. 1980; Collatz et al. 1992; Long et al. 1996; Long and Bernacchi 2003). With the gas-exchange technique, the rate of CO₂ assimilation is determined from the change in the CO₂ mole fraction in a chamber or by the eddy covariance method (Long et al. 1996). It is nearly instantaneous and nondestructive, and allows measurement of the total carbon gain by a plant leaf, which can then be extrapolated to the whole plant or stand (Long et al. 1996). As environmental stressors generally reduce CO₂ assimilation, the gas-exchange technique has been applied extensively to study plant responses under stressed conditions (Ciompì et al. 1996; Lima et al. 1999; Pereira et al. 2000; Mielke et al. 2003; Huang et al. 2004). Some studies have also shown that a pronounced decline in gas exchange was the first response to acute stress due to stomatal closure, and that the damage to PSII, reflected in reduced quantum yields, occurred later...
Impacts of Oil Spill Disasters on Marine Habitats and Fisheries in North America

(Eastman and Camm 1995; Guidi et al. 1997; Souza et al. 2004). Thus, measurements of leaf gas-exchange complement data obtained by the chlorophyll fluorescence method for quantifying photosynthesis. In our study, by measuring photosynthesis under field conditions at oil-impacted and control treatments simultaneously, one can determine whether the oiled plants are under additional stress compared to uninjured controls.

The photosynthesis data collected with the PAM/LI-COR instruments and field observations suggested a faster recovery in plants at MP, where oil was removed by physical (waves/erosion) processes, than in plants at HI, where there was little physical removal of oil from the quiescent lagoon. The photosynthesis results from these two locations indicate a different recovery trajectory after oiling, with plants at MP showing recovery to control rates within less than 6 months. In contrast, the plants at HI still exhibited signs of depressed photosynthesis even 1 year after oiling, and contaminant sampling indicated that oil persisted in the sediments and on standing detritus at this location. It is likely that plant photosynthesis at HI may take up to 2 years or longer to fully recover to pre-spill rates, which requires further monitoring.

One of the potential difficulties with the method used to measure photosynthesis is the problem of scaling up from leaf-level measurements to whole-plant stress (Krause and Weis 1991; Long et al. 1996; Maricle et al. 2007). One solution to this problem is to collect many data points on an individual and determine whole-plant stress from the resulting distribution of the data. To ascertain the stress condition of a population of individuals, many similar measurements are required before the underlying pattern can emerge. Luckily, the chlorophyll fluorescence measurements are very rapid and it is easy to obtain many measurements at a site in a short period, dark-adaptation time required for PQY measurements notwithstanding. This is not the case for the gas-exchange measurements with the LI-COR, where a single P–I curve took as many as 20–30 minutes to complete. Therefore, the gas-exchange technique requires much more time to collect a statistically robust data set, reducing the investigator's ability to obtain sufficient samples to determine population-level condition with a high degree of confidence. As photosynthesis is the initial process that drives the subsequent increase in biomass and shoot density over time, it can be used as an early indicator of recovery. In this study, we found that smooth cordgrass photosynthesis recovered rapidly on a high-energy eroding shoreline, even after heavy oiling, suggesting a high potential for recovery of shoot density and biomass, two variables that are more routinely collected (Lin and Mendelssohn 1996, 1998; Michel et al. 2009).

Compared with other studies of DWH oiling on smooth cordgrass habitats, our findings from MP are in agreement with results reported from exposed coastlines by Mendelssohn et al. (2012) and Mishra et al. (2012), however, in heavily oiled and wave-protected areas recovery was proceeding at a slower rate (Silliman et al. 2012) analogous to our findings at HI. Monitoring for recovery from oil impacts should ideally involve both photosynthesis and growth measurements, but growth is slower to respond than photosynthesis.

An additional caveat that is important to consider in any photosynthesis monitoring is that seasonal changes in plant photosynthesis rates may be misinterpreted as chronic oil stress effects. In the data collected over the course of 1 year, the seasonal dormancy of smooth cordgrass was clearly evident as depressed photosynthesis during the winter months (Figure 7.12). It was important, therefore, to have data from adjacent unimpacted control treatments at each location to properly interpret whether depressed photosynthesis was a function of oil pollution or whether it was a response to the low temperatures and reduced day-lengths that induce winter dormancy in this species. Failing to recognize this important natural process could easily have been misinterpreted as a chronic depression due to oiling, which would have been incorrect (compare Figures 7.1 and 7.12).
During the winter months, the contrast between the control and the two impacted locations in the field data was not as obvious as during the growing season earlier in 2010, which could be explained either by complete recovery of photosynthesis from oil impacts or by seasonally lower air temperature that is a limiting factor keeping photosynthesis uniformly low. To better understand the interplay of recovery from oil impacts and seasonality on the photosynthesis dynamics, we developed a Hierarchical Bayesian (HB) model to simulate these two sources of plant stress from the field data obtained at MP in a closely related study (see Wu et al. [2012] for details on the model development and interpretation). The results from this model suggest that the photosynthesis rates at the heavily oiled treatment recovered to the status of the control treatment at MP in about 140 days (4.7 months), which was in early December 2010. On the other hand, the oil impact was never severe enough to make the photosynthesis rates at the medium impact treatment significantly lower than those at the control treatment (Wu et al. 2012). This HB model allowed us to better interpret the dynamic interplay of photosynthesis recovery from oil stress and the opposing seasonal depression of photosynthesis as the plants went into winter dormancy, and then to correctly interpret the time to recovery as being within 6 months, even at the heavily oiled treatment at MP. Other recent studies also show rapid recovery in marshes in Louisiana, especially in high-energy coastlines where oil contamination was rapidly diluted and removed (McCall and Pennings 2012; Silliman et al. 2012). The results of these and our study suggest that salt marshes can be very resilient to even heavy oil contamination and that photosynthesis and growth can recover quickly, within less than 1 year, if oil contamination is rapidly reduced by tidal and wave flushing.

![Diagram](image-url)
Conclusions

Oil persisted in salt marsh environments for at least 1 year after arrival. High-energy coastlines, such as MP, allowed more rapid removal or degradation of the oil contamination. Plant recovery was more rapid in these locations based on photosynthesis measurements. In contrast, at low-energy locations with long residence times, such as HI, oil was still detected in sediments and on plants 1 year later. Plants in these locations exhibited chronic stress, which depressed photosynthesis, despite a full year of recovery. As many of the salt marsh coastlines in the northern GOM are eroding, the effects of DWH oil contamination are likely to be short-term (1–3 years), with oil residues being resuspended back to the shallow coastal waters adjacent to the coastline. The implications of these findings on salt marsh function are that along exposed shorelines, removal of oil contaminants by tidal and wave action helps to speed up recovery of photosynthesis in smooth cordgrass, whereas in protected lagoonal shorelines, oil contamination tends to persist in the sediments and may result in protracted recovery.

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References


Oil Contamination in Mississippi Salt Marsh Habitats

Impacts of Oil Spill Disasters on Marine Habitats and Fisheries in North America


Oil Contamination in Mississippi Salt Marsh Habitats

169


