

Oiling rates and condition indices of shorebirds on the northern Gulf of Mexico following the Deepwater Horizon oil spill

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ABSTRACT. The coastline of the Gulf of Mexico in the United States is an important wintering and stopover region for migratory shorebirds. The Deepwater Horizon oil spill (April–August 2010) impacted more than 1700 km of this coastline and could potentially affect shorebirds through long-term exposure to toxins, degraded habitats, and altered food chains. We investigated the exposure to Deepwater Horizon oil of seven species of shorebirds that winter or stopover along the northern Gulf of Mexico. From October 2010 to May 2012, we captured and banded 691 shorebirds at six sites that experienced varying levels of oil contamination. Of birds sampled, 22 were lightly oiled, with species that forage on the coast having higher rates of oiling than those that forage in more estuarine habitats. Although only 8.6% of birds captured from October 2010 to May 2011 and 0.6% of the birds captured from August 2011 to June 2012 showed signs of oiling, an unknown, but potentially larger, number of shorebirds were likely exposed to indirect effects of the spill, such as decreased foraging time due to oiling of sites or disturbance from cleanup activities. Fuel stores and fattening rates of Dunlins (*Calidris alpina*) during spring migration, as measured using plasma metabolites, were not influenced by site oiling level. However, the level of disturbance at study sites was a significant predictor of both fuel stores and glycerol levels, suggesting that Dunlins stopping over during spring migration may have had difficulty reaching necessary fuel stores in spring 2011 due to disturbance from cleanup activity on oiled beaches. These effects from disturbance were only observed at sites with high cleanup activity, suggesting that the impact of oil-spill cleanup on shorebirds may be minimized by limiting cleanup activities to specific areas and times of day.

RESUMEN. Tasa de empetrolamiento e índices de condición de aves costeras en el norte del Golfo de México tras el derrame de petróleo de la Deepwater Horizon.

La costa del Golfo de México en los Estados Unidos es una importante región de invernada y de puerto intermedio para aves costeras migratorias. El derrame de petróleo de la plataforma Deepwater Horizon (Abril – Agosto 2010) impactó más de 1700 km de su costa y puede potencialmente afectar a las aves costeras a través de la exposición prolongada a toxinas, ambientes degradados, y cadena alimenticia alterada. Investigamos la exposición al petróleo de la Deepwater Horizon por parte de siete especies de aves costeras que invernan o tienen paradas intermedias a lo largo del norte del Golfo de México. De Octubre 2010 a Mayo 2012, capturamos y anillamos 691 aves costeras en seis sitios que mostraban niveles variables de contaminación por petróleo. De las aves muestreadas, 22 estaban ligeramente empetroladas, con las especies que forrajean en la costa mostrando las tasas más altas de empetrolamiento respecto a las que forrajean en ambientes más estuarinos. Aún cuando solo el 8.6% de las aves capturadas entre Octubre de 2010 y Mayo de 2011 y el 0.6% de las aves capturadas entre Agosto de 2011 y Junio de 2012 mostraron señales de empetrolamiento, un número desconocido pero potencialmente mayor de aves costeras han sido posiblemente expuestas a efectos indirectos del derrame, como ser disminución en el tiempo de forrajeo debido a empetrolamiento de sitios o disturbios por actividades de limpieza del sitio. Los depósitos de energía y la tasa de engordamiento del Playero común (*Calidris alpina*) durante la migración de primavera, mediada usando metabolitos plasmáticos, no se vieron influenciados por el nivel de empetrolamiento del sitio. Aún así, el nivel de disturbio en el sitio de estudio fue un predictor significativo de tanto los depósitos de energía como de los niveles de glicerol, sugiriendo que los Playeros comunes que se detienen durante la migración de primavera pueden haber tenido dificultades en alcanzar depósitos de energía necesarios en primavera del 2011 debido a disturbios por actividades de limpieza en playas empetroladas. Aún así, los efectos de los disturbios fueron solo observados en sitios con alta actividad de limpieza, sugiriendo que el impacto de limpieza de derrame de petróleo en aves costeras puede ser minimizado limitando las actividades de limpieza a áreas y horarios específicos.

Key words: disturbance, fuel load, migration, refueling rate, plasma metabolites

The beaches and wetlands of the northern Gulf of Mexico (hereafter GOM) are important

habitats for 34 species of shorebirds (Withers 2002). During the nonbreeding season, these habitats host more than 5% of the total North American population of 12 species of migratory

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shorebirds (Henkel et al. 2012). Habitats of the northern GOM face persistent threats from both environmental and anthropogenic change. Louisiana, for example, is experiencing the most severe land loss and barrier island erosion in North America (Penland and Ramsey 1990), due in part to rising sea levels (Miner et al. 2009). Other threats include habitat loss due to shoreline development and erosion. Potential threats to shorebird populations using these vulnerable habitats were compounded when the Deepwater Horizon (hereafter DWH) oil platform exploded and sank on 20 April 2010, resulting in the release of 4.9 million barrels of crude oil (McNutt et al. 2012). Some of this oil washed up on the beaches and marshlands of the northern GOM, subsequently stranding oil on 1773 km of coastline (Michel et al. 2013). Shorebirds were particularly vulnerable to this event because they forage in readily oiled, intertidal shoreline habitat (Peterson et al. 2003, OSAT-2 2011).

The effects of oil on birds have been well documented (Leighton 1993, NRC 2003, Henkel et al. 2012). Extreme cases can include mortality due to a loss of water-proofing or to ingested oil, which may lead to toxicological effects, including hemolytic anemia, immuno-suppression, and liver and kidney damage (Leighton 1993, Briggs et al. 1996). Although exposure to oil is not necessarily fatal, even limited exposure can negatively impact birds by reducing foraging success, either through a loss or degradation of food resources (Andres 1997, NRC 2003) or by reducing foraging time because of the need to preen oiled feathers (Burger 1997, OSAT-2 2011). Clean-up activities following an oil spill can also increase disturbance and reduce foraging time for shorebirds. For example, following the Anitra oil spill off the New Jersey coast in 1996, Burger (1997) observed a significant reduction in foraging time and an increase in time spent foraging by shorebirds due to cleanup activities.

To understand the ongoing impacts of the DWH spill, methods are needed to measure long-term, sub-lethal effects. One method is to estimate mean energy stores of birds at stopover sites, measured through size-adjusted mass. Because large fuel stores are necessary for shorebirds departing on long-distance migrations (Warnock 2010), mean energy stores have been used to monitor changes in habi-

tat quality at shorebird migration sites (Baker et al. 2004, Hicklin and Chardine 2012, Mizrahi et al. 2012).

Another potential method is to measure plasma metabolites. During spring, shorebirds minimize time spent migrating (Lyons and Haig 1995, Farmer and Wiens 1999) in an effort to quickly reach northern breeding grounds and establish breeding territories. One of the primary factors determining migration speed and success is the efficiency of refueling rates at stopover sites (Alerstam and Lindstrom 1990). Fattening rates of birds during migration may therefore be used as a proxy for habitat quality when comparing stopover sites because high refueling rates allow birds to continue migration faster. Plasma metabolites have been shown to be useful indicators of fattening rates in a variety of shorebird and passerine species (Jenni-Eiermann and Jenni 1994, Williams et al. 1999, Guglielmo et al. 2005, Seaman 2005). Because levels of some plasma metabolites, such as triglycerides, increase during fat deposition and others (e.g., glycerol and β -hydroxybutyrate) increase during fat catabolism, metabolites can provide an instantaneous measure of the physiological state and energetic condition of birds. Plasma metabolites have been used as measures of habitat quality in a variety of studies where correlations between fattening rates, resource abundance, and availability of foraging habitat have been observed (Guglielmo et al. 2005, Seaman 2005, Seewagen et al. 2011). For example, Seaman et al. (2006) found that Western Sandpipers (*Calidris mauri*) using stopover sites with shorter mudflat exposure times (i.e., decreased foraging opportunities) had lower triglyceride levels during northward migration.

Different species of shorebirds use different habitats and microhabitats, facilitating assessment of contamination from oil across a coastal landscape. Sanderlings (*Calidris alba*), for example, forage primarily along tidal beach fronts (Burger and Gochfeld 1991), whereas other sandpiper species spend more time in estuaries or bayside wetlands (Holmes 1972, Baker and Baker 1973). Within habitats, shorebirds differ in their foraging behavior. Least Sandpipers (*C. minutilla*) tend to forage in brackish marshes and along the inner edge of mudflats between clumps of marine vegetation (Nebel and Cooper 2008, Mizrahi et al. 2012), whereas other estuarine species are more commonly found in open

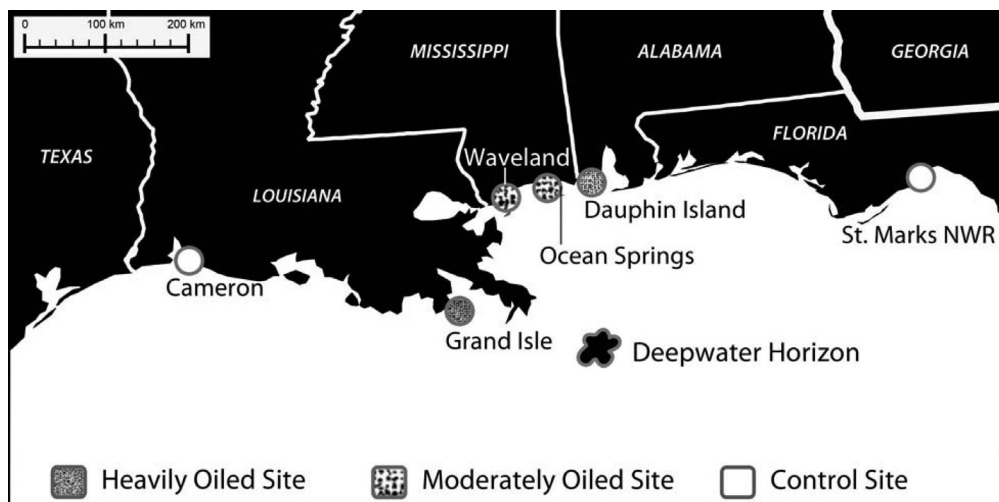


Fig. 1. Location of and levels of maximum oiling at study sites on the northern Gulf of Mexico.

mudflat habitats (Wilson 1994, Hicklin and Gratto-Trevor 2010). Coastal wetland habitats used by shorebirds on the northern GOM are primarily barrier islands (Withers 2002) where Gulf-facing beaches are abutted by dunes, and the bayside/central portions of the islands consist of vegetated mudflats (Rosati and Stone 2009). These habitats experienced varying levels of oiling from the DWH oil spill. Gulf-side habitats were more directly impacted by oil, but oil was also deposited in bayside habitats and vegetation by waves (Michel et al. 2013). Chapman (1984) investigated the vulnerability of a variety of shorebird species to oil spills following the Ixtoc I oil spill along the Texas GOM coast, and found that birds foraging in shoreline habitats of barrier islands were more vulnerable to oil exposure than those using the backshore.

We examined the potential indirect effects of the DWH oil spill on several species of migratory shorebirds and one species of resident shorebird over a 2-yr period on the northern GOM. Because birds can be impacted by even a small amount of oil, our objective was to expand on surveys conducted by the Natural Resource Damage Assessment to monitor oiling rates across species (NRDAR 2012) by examining birds in the hand so even trace amounts of oil could be detected. Our specific objectives were to quantify exposure across habitats and species with a variety of foraging behaviors, and to assess

the degree to which variation in fuel stores and refueling rates could be explained by oiling and disturbance rates.

METHODS

Study sites. Beginning in October 2010, birds were captured and banded at six sites along the northern GOM (Fig. 1). These sites represented four habitat types commonly found on the GOM coast, including mudflats, back bays, sandy beaches, and remnant wetlands. These sites were selected because they host shorebird populations throughout the year and were monitored by the Audubon Coastal Bird Survey (Johnson 2011).

Site oiling level. The oiling level of each site was classified using maximum oiling categories (MAX) across 2010–2012 from Shoreline Cleanup Assessment Technique (SCAT) data from the National Oceanic and Atmospheric Administration, as well as by oiling levels following cleanup activities according to SCAT surveys from May 2011 and May 2012 (hereafter, post-cleanup oil level or PC; Michel et al. 2013, Table 1).

Site disturbance level. The disturbance level of each site was categorized by the average number of times foraging shorebirds (both target and non-target species) were interrupted during 30-min point count surveys conducted using the International Shorebird Survey Protocol

Table 1. Habitat, oiling, and disturbance characteristics of study sites on the northern Gulf of Mexico.

Site	Site area (m ²)	Habitat	Substrate	Sampling period	Number of surveys	Maximum oiling level ^a	SCAT oiling level, May 2011	SCAT oiling level, May 2012	Disturbance level, 2011 ^b	Disturbance level, 2012
Broussard's Beach, Cameron, LA	0.65	Mudflat	Mud and fine-grained sand	December 2010–June 2012	10	None	None	None	Low (no cleanup)	Low
Elmer's Island, Grand Isle, LA	0.28	Backbay, Remnant wetland	Mud, fine-grained sand with vegetation	October 2010–June 2012	11	Heavy	Heavy	Light	High (mechanical cleanup)	Medium (manual cleanup)
Waveland Beach, Waveland, MS	0.03	Sandy beach	Coarse sand	December 2010–May 2011	9	Moderate	Very light	Very light	High (mechanical cleanup)	N/A
Graveline Bayou, Ocean Springs, MS	0.03	Remnant wetland	Mud with vegetation	December 2010–June 2012	10	Moderate	Light	Very light	Low (no cleanup)	Low
West End Beach/Pier, Dauphin Island, AL	0.15	Sandy beach	Coarse sand	January 2011–June 2012	9	Heavy	Very light	Very light	Medium (manual cleanup)	Medium (public beach open)
St. Marks National Wildlife Refuge, St. Marks, FL	0.43	Backbay	Mud and fine-grained sand	December 2010–February 2011	5	None	None	None	Low (no cleanup)	N/A

^aOiling level for each site is classified using maximum oiling rates across 2010–2012 from Shoreline Cleanup Assessment Technique (SCAT) data from the National Oceanic and Atmospheric Administration (Michel et al. 2013).

^bHigh disturbance = birds disturbed > 4 times during 30-min point counts (mechanical cleanup activity); Medium disturbance = birds disturbed three to four times (non-mechanical cleanup activity and well populated beaches); Low disturbance = birds disturbed <3 times (primarily from people walking, sometimes with dogs).

(Manomet 2010). An interruption was counted if birds stopped foraging and flew $> \sim 30$ m. Disturbance levels were categorized as low (0–2 times), medium (3–4 times), and high (> 4 times; Table 1). During fall 2010–spring 2011, high disturbance levels were only observed at sites with mechanical oil spill cleanup activities taking place. Mechanical cleanup included use of bulldozers for tar mat excavation, mechanical sand sifters (OSAT-2 2011), and > 10 all-terrain vehicles (> 50 individuals on the beach at least four days a week between 06:00 and 17:00; JRH, pers. obs.). Medium disturbance was associated with either manual cleanup activities, where workers were removing oil from intertidal sand with shovels and rakes (OSAT-2 2011), or disturbance from activities on public beaches (including people and dogs).

Surveys. Surveys were conducted at the same sites as mist-net sampling (see Table 1) between 07:00 and 09:00 or 15:00 and 17:00 on or within one day of mist-netting. However, surveys were not conducted while mist-nets were set because that could have increased disturbance. Disturbance surveys were conducted in foraging areas with a minimum of 15 foraging birds present, although the median shorebird number during these surveys was 125. Foraging areas in habitats on the northern GOM are highly localized, resulting in small areas with large numbers of birds foraging (Table 1).

Other data collected. In addition to disturbance level, other data collected during surveys included time of day, tidal height (according to nearest National Data Buoy; NDBC 2014), survey area, and predator abundance. Raptors, particularly falcons and accipiters, are the main predators of shorebirds during the non-breeding season (Colwell 2010). We evaluated the effect of predation pressure at our study sites during sampling by counting the daily mean number of falcons (accipiters were not detected) observed during surveys days and trapping sessions (maximum number observed on any single day was 2, a single Merlin [*Falco columbarius*] and a single Peregrine Falcon [*F. peregrinus*]).

Study species. At our six study sites, we evaluated the oiling status of one resident shorebird (Wilson's Plover, *Charadrius wilsonia*) and six species of migrating shorebirds, including Sanderlings, Dunlins (*Calidris alpina*), Semipalmated Plovers (*Charadrius semipalmatus*), Western Sandpipers, Least Sandpipers, and

Semipalmated Sandpipers (*C. pusilla*). Sanderlings primarily forage along Gulfside beaches (Burger and Gochfeld 1991). Dunlins and Semipalmated Plovers also forage along tidal beachfronts and are commonly found in back-bay mudflat habitats. Least, Western, and Semipalmated sandpipers forage largely in estuaries or in back-bay habitats, and rarely on Gulfside beaches (Holmes 1972, Baker and Baker 1973). Least Sandpipers preferentially forage along the edges of mudflats between clumps of marine vegetation (Nebel and Cooper 2008), and Western and Semipalmated sandpipers are more commonly found in open mudflat habitats (Wilson 1994, Hicklin and Gratto-Trevor 2010). We selected Wilson's Plovers as our target resident species because they are a species of high conservation concern (Brown et al. 2001) that foraged and nested in coastal habitats while the DWH well was still leaking.

For Dunlins, we also measured mean fuel stores (size-adjusted mass) and refueling rates (as indicated by plasma metabolite levels) during spring migration (March–May) and compared the results for spring 2011 and spring 2012. Dunlins were chosen for this analysis because they are believed to use a “jump” migration strategy in the spring, making long migratory flights across the central United States from wintering or stopover sites on the northern GOM en route to near-Arctic breeding grounds (Skagen et al. 1999). Because these long flights require large fuel stores, habitat quality of stopover sites on the northern Gulf may be especially important. Dunlins captured during fall migration were not included in these analyses because evidence suggests that shorebirds tend to exhibit time minimization behavior during spring migration (Lyons and Haig 1995, Farmer and Wiens 1999) in an effort to reach breeding grounds as soon as possible. Such constraints are relaxed during fall migration and, in some shorebird species, migrants tend to limit fat reserves, suggesting an energy-minimization strategy (Gudmundsson et al. 1991, Lyons and Haig 1995).

Bird sampling. Beginning in October 2010, shorebirds were captured at our six study sites (Table 1). Birds were captured primarily at dawn and dusk using six mist-nets placed in foraging areas for 3–5 h. Birds were passively captured in nets that were monitored constantly so birds were retrieved from nets within

5–10 min. Captured birds were weighed (± 0.1 g) using a digital balance, measured (wing chord, tarsus length, and exposed culmen), and banded with a USGS numbered band. Each bird was classified as adult or juvenile based on plumage characteristics (Prater et al. 1977), and fat scores were measured on a scale from 0 to 5 (Helms and Drury 1960). The level of oiling on each bird was measured using the Natural Resource Damage Assessment and Restoration (NRDAR) protocols, where birds are categorized as (1) not visibly oiled, (2) trace amounts of oiling (<5% of total body area covered with oil), (3) lightly oiled (5–20%), (3) moderately oiled (21–40%), and (4) heavily oiled (>40%; NRDAR 2010). We assessed mean fuel stores of Dunlins using an index of size-adjusted mass that adjusts body mass for size using the volumetric conversion of wing chord, that is, size-adjusted mass = mass/(wing chord)³ \times 10,000 (Winker et al. 1992, Mizrahi et al. 2012, Hicklin and Chardine 2012). Fat scores of Dunlins (corrected for Julian date) were significantly correlated with size-adjusted mass ($F_{1,184} = 50.8$, $P < 0.001$), supporting our use of this as a measure of energetic condition. We present our results for size-adjusted mass instead of fat scores due to the subjective nature of fat scoring measurements.

Blood sampling and plasma metabolite analyses. To assess refueling rates of Dunlins, we collected blood samples (200–300 μ l) from the brachial vein using a 26-gauge needle and heparinized capillary tubes. Blood samples were stored on ice in the field and centrifuged within 2 h at 6000 rpm for 10 min. Plasma samples were stored in a -20°C freezer for 1 to 2 weeks and then stored at -80°C prior to analysis.

Lipid metabolites were assayed in 400- μ l flat-bottom 96-well microplates (Greiner Bio-One, Monroe, NC) in a microplate spectrophotometer (340EL, BioTek, Winooski, VT; Guglielmo et al. 2005, Williams et al. 2007). Due to small plasma volumes, not all metabolites could be determined for all individuals. Following previous studies (Schaub and Jenni 2001, Guglielmo et al. 2002, 2005), we prioritized triglyceride and glycerol assays. Total triglyceride (TRIG) and free glycerol (GLYC) were measured by endpoint assay (Sigma Diagnostics Trinder reagents A and B, Sigma-Aldrich, St. Louis, MO; Williams et al. 1999). True triglyceride concentration (mmol L^{-1}) was calculated by subtracting glycerol from total

triglyceride. Inter-assay coefficients of variation (CV) based on chicken plasma pools were 8.4% for TRIG and 9.9% for GLYC ($N = 9$ assays over 2 yr). β -Hydroxybutyrate (BOH) was measured by kinetic endpoint assay (kit E0907979, Biopharma, Lexington, MA; Guglielmo et al. 2005). The inter-assay CV for BOH was 13.4% ($N = 9$). All samples were run in duplicate or triplicate. Intra-assay CVs for TRIG, GLYC, and BOH 2011 were 6.2%, 4.4%, and 13.6%, respectively. Intra-assay CVs in 2012 were 3.6% for TRIG, 2.8% for GLYC, and 8.8% for BOH.

Statistical analyses. Fisher's exact test was used to test for differences in oiling rates between species and foraging habitats (coastal or back bay). We assessed the effects of site oil level (according to maximum oiling level and SCAT oiling levels in May 2011 and 2012) and site disturbance activity using general linear models with a backward selection procedure ($\alpha = 0.1$) to identify independent variables that contributed to differences among sites. Size-adjusted mass and metabolite concentrations were $\log_{10} + 1$ transformed to normalize the data. Independent variables of Julian date, time of day, tidal height, bleed time (time between capture and bleeding), mean predator abundance, size-adjusted body mass, and habitat type were assessed. We compared metabolite concentrations and size-adjusted mass by site oil level or disturbance level with analysis of covariance (ANCOVA), with variables retained by the general linear model entered as covariates (Guglielmo et al. 2005). Results of the reduced models are presented, including significant covariates. We made all pairwise post-hoc comparisons using the Tukey's HSD test. We did not assess variation in disturbance levels between years because disturbance level was categorized (as described above) for individual sampling occasions (i.e., it varied within years and within sites). Although significant sex effects in stopover ecology have been reported in other studies (Warnock and Bishop 1998, Bishop et al. 2005, 2006), effects of sex or age have not been consistent across studies. For example, Guglielmo et al. (2002) and Seaman et al. (2006) reported no sex or age differences in triglyceride levels. For our analyses, samples were pooled across ages and sex due to small sample sizes. Values are presented as least-square means \pm SE. All statistical analyses were conducted using R Software version 0.97.551 (R Core Team 2013).

RESULTS

Oiling rates across species and sites.

From October 2010 to May 2012, we captured 691 shorebirds, with 76 captured during fall/winter 2010–2011, 146 during spring 2011, 37 during fall/winter 2011–2012, and 432 during spring 2012. Of birds sampled, 22 were visibly oiled. Most oiled birds (17/22) were captured at sites that were heavily oiled (Fig. 2A and B). Oiled birds showed only light (2/22) or trace (20/22) amounts of oiling. From October 2010 to May 2011, 8.6% of captured birds (19/222) were oiled, and the percentage decreased to 0.6% (3/469) during the period from August 2011 to May 2012. Overall, 3.2% (22/691) of birds were oiled.

Among migratory species, Sanderlings had the greatest proportion of oiled individuals (5% of total captured; Fig. 2C), followed by Dunlins (4.2%), Semipalmated Plovers (2.4%), and Least Sandpipers (2.3%). No Semipalmated or Western sandpipers were visibly oiled (Fig. 2C). Seven of 16 Wilson's Plovers (43.8%) had visible signs of oil (Fig. 2C). Oiling rates differed between foraging habitats (coastal vs. estuarine; Fisher's exact test, $P = 0.001$).

Residual size-adjusted mass and plasma

metabolite levels. Size-adjusted mass was compared for 184 Dunlins captured during spring migration in 2011 and 2012. In multiple regression analyses of significant covariates, Julian date was the only significant predictor of mass across sites. Controlling for date, residual size-adjusted mass across years did not differ with either maximum ($F_{2,181} = 0.2$, $P = 0.8$) or post-cleanup oiling levels ($F_{3,180} = 2.0$, $P = 0.12$). Mean residual mass did not differ among either maximum or post-cleanup oil levels within years (all $P \geq 0.10$, Fig. 3A). Across years, residual mass differed with disturbance level ($F_{2,181} = 4.8$, $P = 0.01$), with Dunlins having lower residual mass at sites with high levels of disturbance than at sites with low (Tukey's HSD, $P = 0.04$) or medium ($P = 0.007$) levels. Residual mass of Dunlins did not differ between sites with medium and low levels of disturbance (Tukey's HSD, $P = 0.77$; Fig. 3A).

Plasma metabolite TRIG concentrations of 184 Dunlins were measured during spring 2011 and 2012. We found no relationship between TRIG and site maximum oiling level across or

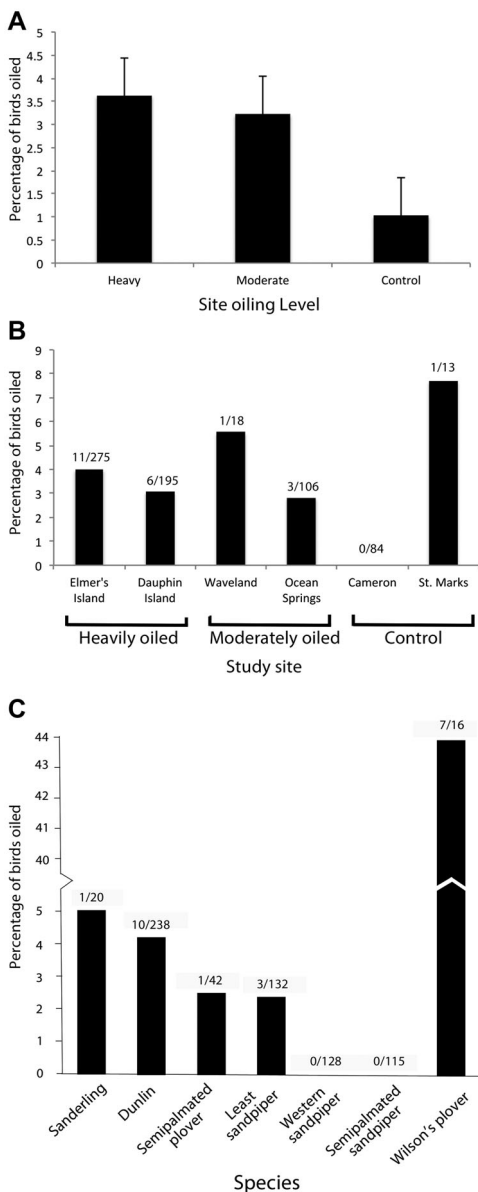


Fig. 2. Proportions of total oiled shorebirds observed (A) across study sites, (B) by maximum site oiling level, and (C) by species. Numbers above bars indicate number of oiled birds relative to the total number captured.

within years ($F_{2,181} = 1.2$, $P = 0.30$ across years; 2011: $F_{2,51} = 0.03$, $P = 0.97$ for 2011, 2012: $F_{2,127} = 1.7$, $P = 0.18$, controlling for Julian date and size-adjusted mass, with no other covariates significant in the full model; Fig. 3B). There

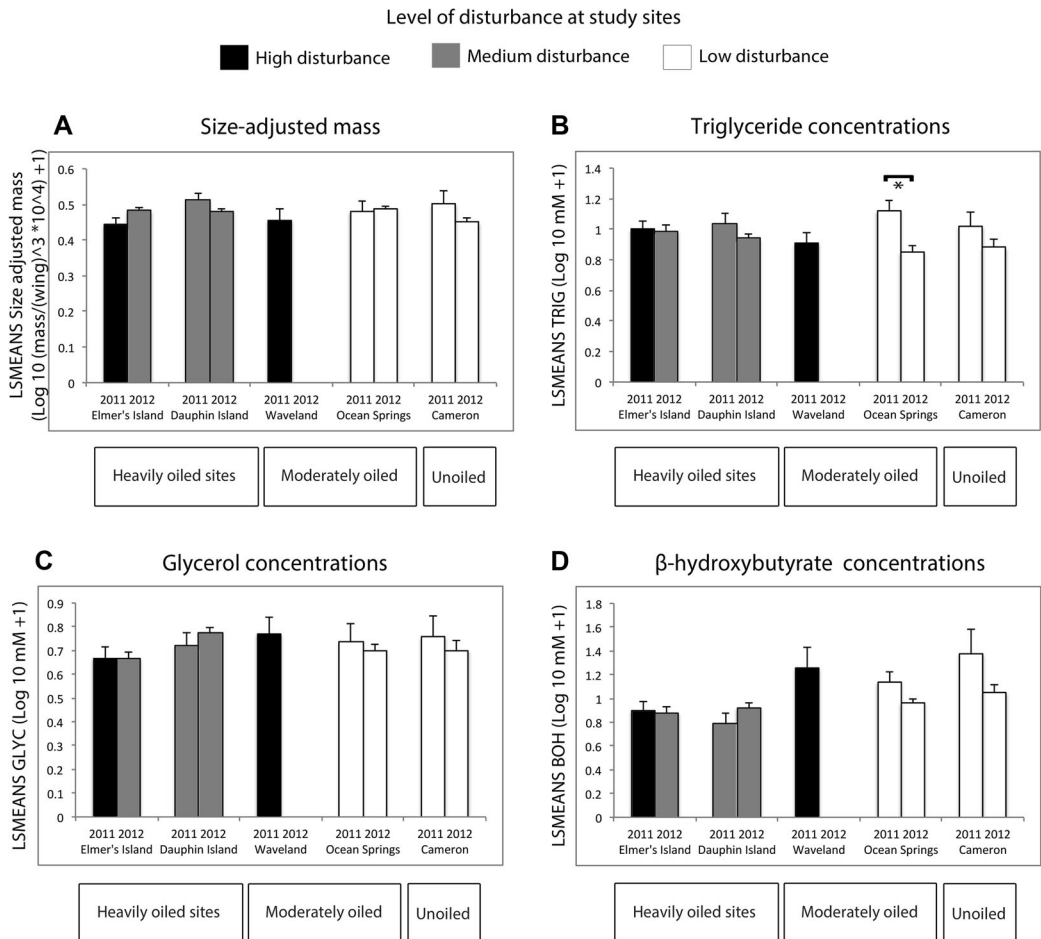


Fig. 3. Size-adjusted mass and plasma metabolite concentrations of spring-migrating Dunlins during 2011 and 2012 across study sites on the northern Gulf of Mexico. (A) Residual size-adjusted mass (mass/[wing chord]³ × 10,000) was significantly lower at high-disturbance sites than at low- or medium-disturbance sites. (B) Residual Triglyceride levels showed no difference based on oil or disturbance status, but there was a significant difference between years at Ocean Springs. (C) Residual glycerol concentrations were significantly lower at lightly oiled sites than at very lightly oiled sites, and significantly higher at medium-disturbance sites than at low-disturbance sites. (D) Residual β-hydroxybutyrate levels did not differ based on oil or disturbance status. Values are least-squared means ± SE.

was also no relationship between TRIG and post-cleanup oil level across years ($F_{3,180} = 1.5$, $P = 0.21$) or within years (2011: $F_{3,50} = 1.0$, $P = 0.40$; 2012: $F_{2,127} = 1.4$, $P = 0.25$). Residual TRIG concentrations were not influenced by level of disturbance across years ($F_{2,181} = 0.8$, $P = 0.45$).

GLYC levels of Dunlins differed with both maximum and post-cleanup site oiling level across years ($F_{2,181} = 3.3$, $P = 0.04$ for MAX;

$F_{3,180} = 2.7$, $P = 0.05$ for PC), after correcting for Julian date and time of day. GLYC levels at “lightly” oiled sites were lower than levels at “very lightly” oiled sites (Tukey’s HSD, $P = 0.04$). No other post-hoc pairwise comparisons for MAX or PC showed significant differences. Within years, we found no difference in GLYC levels among either maximum (2011: $F_{2,52} = 0.2$, $P = 0.86$, 2012: $F_{2,127} = 2.3$, $P = 0.11$) or post-cleanup (2011: $F_{3,50} = 0.6$, $P = 0.64$, 2012:

$F_{2,127} = 1.8$, $P = 0.18$, Fig. 3C) oil levels. GLYC levels of Dunlins differed with site disturbance level across years ($F_{2,181} = 3.3$, $P = 0.04$). Post-hoc pairwise comparisons revealed higher GLYC levels at medium- than at low-disturbance sites (Tukey's HSD, $P = 0.02$), but the difference in mean GLYC concentrations between high- and low-disturbance sites was not significant ($P = 0.77$; Fig. 3C).

BOH analyses ($N = 95$) were corrected for Julian date, bleed time, and habitat. No differences were observed in BOH levels by site maximum or post-cleanup oiling level either across (MAX: $F_{2,92} = 0.1$, $P = 0.94$, PC: $F_{3,91} = 0.1$, $P = 0.98$; Fig. 3D) or within years (MAX, 2011: $F_{2,11} = 0.1$, $P = 0.91$, 2012: $F_{2,78} = 0.03$, $P = 0.97$; PC, 2011: $F_{2,11} = 0.0$, $P = 1.0$, 2012: $F_{2,78} = 0.02$, $P = 0.98$). Across years, BOH concentrations did not differ with site disturbance level ($F_{2,92} = 0.3$, $P = 0.73$; Fig. 3D).

DISCUSSION

More than 1 million shorebirds migrate through habitats impacted by the Deepwater Horizon Oil spill (Vermillion 2012, Henkel et al. 2012). In two years following the spill, 8.6% (2010–2011) and 0.6% (2011–2012) of shorebirds captured on the northern GOM had light or trace oiling, suggesting that the number of shorebirds potentially affected by oil during those years could have been as high as 100,000 birds (Henkel et al. 2012). Although oiled birds had only light or trace amounts of oil on their feathers, shorebirds with less than 15% of their body area covered with oil may have significantly reduced feeding times (Burger 1997). The number of oiled birds in our study may not have been as high as expected given the scale of the Deepwater Horizon Oil spill, and one possible explanation for this is that we did not begin our study until October 2010, almost six months after the start of the spill. Additionally, our study sites were located where birds were foraging, and not necessarily where oiling levels were high. We did not expect to capture moderately or heavily oiled birds because they would probably either be too ill to forage or would have already perished.

Our results suggest that species of shorebirds using the outer coast, such as Sanderlings and Dunlins, were more likely to be oiled than more estuarine sandpipers, such as Western or Semipalmated sandpipers. Of 88 live shorebirds

and shorebird carcasses captured or collected by USFWS personnel following the DWH spill, Sanderlings ($N = 26$) were the most common (4 visibly oiled, 20 not visibly oiled, and 2 with unknown oiling levels; USFWS 2011). Such microhabitat variation in oiling rates of birds is not surprising as many Gulf-side habitats experienced higher oiling rates than bayside estuarine habitats (Michel et al. 2013).

Although they are not found in high numbers during the non-breeding season in areas impacted by oil (Zdravkovic 2013), we found that a relatively high percentage of resident Wilson's Plovers had visible signs of oiling (43% of birds captured). This suggests that Wilson's Plovers and other resident species that spend most of the year on the northern GOM were more likely to be exposed to oil. In a study of the effects of the Exxon Valdez oil spill on marine bird communities immediately after and during the year following the spill, Wiens et al. (1996) found that birds most impacted (significantly lower populations) were shoreline-feeding species that either breed or winter on beaches or were full-year residents in habitats that were oiled. Similarly, Andres (1997) observed fewer breeding pairs of Black Oystercatchers (*Haematopus bachmani*) and lower nest success in oiled habitats during the first summer after the Exxon Valdez spill. However, by 1991 (three summers after the spill), the breeding population in these areas had substantially recovered (Andres 1997). Like Black Oystercatchers, Wilson's Plovers in our study were nesting during the summer immediately following the spill, and the high oiling levels we observed suggests they, and other resident species of the GOM, may have been particularly negatively impacted by the DWH spill.

We found no evidence that oiling levels affected fattening rates and plasma metabolites of Dunlins. These results suggest that, although some variation in fattening rates may occur from high levels of oil in foraging habitat, on average, birds were fattening at the same rate regardless of the amount of oil in the environment. Therefore, food resources may have been adequate for attaining relatively high instantaneous refueling rates, even in heavily oiled habitats. One possible explanation for this could be increased abundance of prey in oiled habitats due to agitation of substrates during cleanup activity. Ferns et al. (2000) observed increased numbers

of shorebirds and gulls feeding in intertidal habitats following disruption of sediment by mechanized cockle harvesters in South Wales. They suggested this (temporary) increase might have been due to a decrease in the amount of sand in the upper layers of the sediment, making prey detection or extraction easier. In heavily oiled habitats on the GOM, large machinery was used to dig up tar mats that formed as a result of the spill, and we observed that birds avoided these areas during active cleanup, but on multiple occasions flocks of birds (often >20 individuals) were observed foraging in exposed mats once human activity ceased for the evening (JRH, pers. obs.). Another potential explanation is that birds in our study were only captured while actively foraging. If so, even if refueling rates were diminished due to effects of the oil spill, plasma metabolites would not indicate this because they only provide a snapshot of what the bird was doing at that time. Williams et al. (1999) noted that, although studies of captive Western Sandpipers (*C. mauri*) suggest that metabolites reflect mass change over 1–2 days, plasma metabolite profiles in free-living sandpipers may simply reflect instantaneous rates of mass change, or change over short time periods (0.5–1 day).

Our results indicate that disturbance from oil spill cleanup activities affected shorebirds. Across years, we found that Dunlins had lower energy reserves (as measured by size-adjusted mass) at sites with the highest level of disturbance (where mechanical cleanup was taking place), and lower GLYC concentrations (indicating fat catabolism) at sites with low levels of disturbance. TRIG and BOH concentrations did not differ with site-disturbance level. This suggests birds may be able to maintain high instantaneous fattening rates (i.e., no difference in TRIG levels) at highly disturbed sites, but energy expended to avoid areas with high levels of disturbance may prevent them from attaining large fuel stores. Fuel stores of Dunlins did not differ between sites with medium (non-mechanical cleanup as well as people walking on the beach) and low disturbance, suggesting that shorebirds can tolerate a limited amount of disturbance, but high levels are harmful. The lack of observed differences between medium and low disturbance sites may be the result of habituation to a more reduced human disturbance (Fitzpatrick and Bouchez 1998, Sutherland 2007,

Dwyer 2010) or access to alternative foraging habitats (Gill et al. 2001, Gill 2007). During the demolition and cleanup of a mudflat in Jersey City, New Jersey, Burger (1988) observed lower foraging rates in shorebirds and gulls in the area of highest disturbance activity. However, during this cleanup operation, activity was limited to only 100-m stretches of the beach at a time. Burger (1988) documented birds roosting and foraging on unoccupied areas of the beach, and returning to forage in the cleanup areas once workers left for the day, suggesting that restricting cleanup activity to a limited area allowed birds to continue foraging in undisturbed areas.

Although care was taken by USFWS personnel to minimize the amount of disturbance at oiled sites, at our high disturbance study sites, we observed more than 30 all-terrain vehicles and three to four backhoes traveling up and down several kilometers of beach two to three times per day moving workers and oil-cleanup refuse. Sandpipers spend as much as 70–80% of their time foraging during spring stopover when preparing for migration (Davis and Smith 1998, De Leon and Smith 1999). If foraging is frequently interrupted by disturbance, birds may be delayed in attaining the fuel stores necessary for their long migratory journeys, thus delaying their departure for their breeding grounds (Morrison et al. 2006), which ultimately leads to decreased reproductive success through carry-over effects (Baker et al. 2004, Harrison et al. 2011). The negative impacts of cleanup activities observed in our study might have been minimized by limiting such activity during the height of migration (e.g., April and May) or by limiting cleanup to hours of the day when shorebirds typically roost away from foraging areas.

Our results suggest that disturbance caused by oil cleanup activity may have a greater impact on shorebirds than the oil itself. Although we are not suggesting that cleanup activities should not have occurred following the DWH oil spill, taking into account the appropriate intensity and timing of such activities is important. Our results and those of other investigators suggest that the possible negative impacts of cleanup activities on shorebirds can potentially be minimized by restricting cleanup to specific sections of beach at a time (Burger 1988) or limiting cleanup activities to certain periods of the day (Stillman et al. 2007).

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