Polycyclic aromatic hydrocarbon exposure in seaside sparrows (Ammodramus maritimus) following the 2010 Deepwater Horizon oil spill

Anna A. Perez-Umphrey a,⁎, Christine M. Bergeon Burns a, Philip C Stouffer a, Stefan Woltmann b, Sabrina S. Taylor a

a School of Renewable Natural Resources, Louisiana State University and AgCenter, 227 RNR Building, Baton Rouge, LA, 70803, USA
b Department of Biology, and Center of Excellence for Field Biology, Austin Peay State University, 601 College St., Clarksville, TN, 37040, USA

HIGHLIGHTS

• Is a terrestrial bird responding to contaminated marsh by detoxification metabolism?
• We compared marsh oiling and PAHs in sediment to gene expression in sparrows.
• We used qPCR to examine CYP1A gene expression in sparrows.
• Sparrows in areas with higher PAHs in sediments had elevated CYP1A gene expression.

GRAPHICAL ABSTRACT

ABSTRACT

The seaside sparrow (Ammodramus maritimus) is an abundant and permanent resident of coastal salt marshes impacted by the 2010 BP Deepwater Horizon oil spill. Such terrestrial species are often overlooked in the aftermath of marine spills, despite the potential for long-term oil exposure. We sampled the livers of seaside sparrows residing in oiled and unoiled sites from 2011 to 2014 and quantified expression of cytochrome p450 1A (CYP1A), a gene involved in the metabolism of polycyclic aromatic hydrocarbons (PAHs). In August 2011, CYP1A expression was markedly higher in birds from an oiled site compared to an unoiled site, but differences had disappeared by June 2012. In June 2013, CYP1A expression was elevated compared to 2012 levels on all sites, including those collected from sites that had not been directly oiled during the spill. This rise in CYP1A expression was possibly due to Hurricane Isaac, which made landfall near our sites between the 2012 and 2013 sampling periods. CYP1A expression was significantly attenuated again in June 2014. We also collected sediment samples from the same marshes for a total concentration analysis of PAHs. The PAH concentrations in sediment samples exhibited a similar pattern to the CYP1A data, supporting the link between marsh PAHs and bird CYP1A expression. These results indicate that contamination from marine oil spills can immediately extend to terrestrial ecosystems, and that storms, weather, or other factors may influence subsequent spatial and temporal oil exposure for several additional years.

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⁎ Corresponding author.
E-mail address: apere41@lsu.edu (A.A. Perez-Umphrey).
1. Introduction

The 2010 Deepwater Horizon (DWH) oil spill was one of the largest oil spills in history and released an estimated 700,000 m$^3$ of crude oil into the Gulf of Mexico over three months (Crone and Tolstoy, 2010). A number of short-term ecological impacts common to large oil spills were documented after the DWH spill, including the mortality of thousands of birds (Antonio et al., 2011; Haney et al., 2014), hundreds of sea turtles (Antonio et al., 2011), and dozens of marine mammals (Antonio et al., 2011; Williams et al., 2011). Long-term studies of the effects of this spill on coastal marshes are ongoing (Bergeon Burns et al., 2014; Rabalais, 2014) and, based on what we have learned from other oil spills, measurable effects are expected to persist for decades (Bodkin et al., 2014; Lindeberg et al., 2017; Reddy et al., 2002). Importantly, the DWH spill differed from other large spills by affecting nearly 1700 km of coastal marshes, which are ecologically and economically valuable (Boesch and Turner, 1984; Costanza et al., 1997) and difficult to clean (Michel et al., 2013; Short, 2017; Zengel et al., 2015). This extensive shoreline oiling increased the likelihood for exposure by terrestrial species using near-shore habitats, a group generally underrepresented in oil spill research (Bergeron Burns et al., 2014).

Polycyclic aromatic hydrocarbons (PAHs) are among the most persistent components of oil and can remain in the environment for years, despite weathering and biodegradation on the sediment surface (Short et al., 2008; Turner et al., 2014a). Oil buried in the sediment under hypoxic or anoxic conditions degrades even more slowly and can remain for years in salt marshes (Turner et al., 2014a, 2014b; Lindeberg et al., 2017). For instance, PAHs from the West Falmouth spill, Massachusetts, USA and the Exxon Valdez spill in Prince William Sound, Alaska, USA remained thirty and twenty-six years later, respectively (Reddy et al., 2002; Lindeberg et al., 2017). After the first few years of the DWH spill, the mortality of plants resulted in an accelerated marsh erosion rate and some oiled sediments were thus washed back into the water (Hester et al., 2016; McClennahan et al., 2013). However, DWH source-fingerprinted oil remained in interior marsh areas in 2011 (Ramsay et al., 2014). Clean-up and ecosystem monitoring techniques appropriate for these dense grass habitats are not as well-developed as in other, less complex environments (Zengel et al., 2015). Adding to the complexity of salt marsh-oil interactions, previously silted-over areas may become resuspended during storms or tidal events, allowing months or years to pass between exposure events (Turner et al., 2014b).

The persistence of PAHs in the environment is of concern because of their wide range of negative effects on animals (Douben, 2003). Measuring cytochrome p450s (CYPs) gene activity in vertebrates is a powerful and commonly used method to track exposure to PAHs. Expression of CYPs increases in organisms exposed to PAHs, producing enzymes that metabolize lipophilic PAHs to a water soluble form that may be easily excreted (Williams et al., 2005). Cytochrome p450 1A (CYP1A) up-regulation in the liver is particularly evident when PAHs are present, because a primary function of the liver is detoxification (Bergeon Burns et al., 2014; Esler et al., 2010; Hervé et al., 2010; Nakaya et al., 2009; Short et al., 2008; Whitehead et al., 2012). For example, Barrow’s goldeneyes (Bucephala islandica) from Prince William Sound showed evidence of ongoing PAH exposure for at least eight years after the Exxon Valdez spill (Trust et al., 2000). Double-crested cormorants (Phalacrocorax auritus) demonstrated significant upregulation of CYP1A activity when experimentally dosed with DWH oil (Alexander et al., 2017). The Gulf killifish (Fundulus grandis), a salt marsh resident, had increased CYP1A expression for at least one-year after the spill, and exposure to sediments affected by DWH oil induced a host of detrimental effects that were both physiological (e.g., altered osmoregulation, respiration, immunocompetence) and developmental (e.g., eggs that failed to hatch) (Dubansky et al., 2013; Whitehead et al., 2012). Complementary laboratory studies of Gulf killifish confirmed that the genetic responses observed in the field were consistent with exposure to high concentrations of contaminants in oil (Pilcher et al., 2014), CYP1A expression, as estimated via ethoxyresorufin-O-deethylase (EROD) activity, was also higher in the liver of exposed versus unexposed sea trout (Cynoscion nebulosus) following the DWH oil spill, and was accompanied by higher numbers of splenic melanomacrophage centers, suggesting that exposure to PAHs was coupled to an immune-compromised state (Ali et al., 2014). Organisms frequenting intertidal habitats are particularly vulnerable to long-term, chronic exposure to oil deposited in sediments, lengthening their time to recovery (Esler et al., 2017b). After the Exxon Valdez oil spill, two species that forage in intertidal sediments, the sea otter (Enhydra lutris) and harlequin duck (Histrionicus histrionicus), were especially slow to recover and their exposure to oil in intertidal sediments continued for at least nineteen and twenty-two years, respectively (Bodkin et al., 2012; Esler et al., 2017a).

The seaside sparrow (Ammodramus maritimus) is found from the upper northeastern United States to Florida and the Gulf Coast states, and is one of the few songbirds (Passeriformes) that reside exclusively in coastal salt marshes (Post and Greenlaw, 2009). Because they remain year-round, seaside sparrows rely on the saltmarsh for nesting habitat and foraging grounds, and feed seasonally on seeds, aquatic macroinvertebrates, snails, crabs, and beetle larvae (Post et al., 1983; Post and Greenlaw, 2009). Seaside sparrows exhibit high site fidelity, and their reliance on saltmarsh areas puts them at risk from the combined pressures of flooding, tropical storm systems, and climate change associated sea level rise (Kern and Shrив, 2014; Post and Greenlaw, 2009; Stouffer et al., 2013).

Seaside sparrows are one of the most abundant vertebrates in salt marshes and their continued exposure to oil could be indicative of pervasive contamination in intertidal marsh food webs. Because they frequently forage on the ground, they could come in direct contact with oil, either through consuming oil-exposed prey or foraging on and in contaminated soils. Trace amounts of oil were observed on seaside sparrow feathers soon after the spill (USFWS, 2011) and 14C and 13C analyses showed that there was an incorporation of carbon from oil in sparrow feathers and gut contents (Bonisoli Alquati et al., 2016). Oil could have been ingested while preening oil-covered feathers soon after the spill; however, it is likely that any chronic exposure over several years would occur through lingering oil in sediments that come into contact with seaside sparrows and their prey.

We assessed the magnitude of exposure to PAHs by measuring CYP1A expression in seaside sparrows annually for four years following the DWH oil spill in areas recovering from heavy oiling, as well as in areas that were unoiled in the immediate aftermath of the DWH. We also determined the total PAH concentrations in sediments from the same marshes to estimate oil contamination and to serve as a comparison to CYP1A expression levels.

2. Methods

2.1. Study sites

All study sites were located within Plaquemines Parish, Louisiana. “Oiled” and “unoiled” sites were in Barataria Bay, near Port Sulphur, LA, and “reference” sites were east of the Mississippi near Pointe-a-la-Hache, LA (Fig. 1). Sites were selected based on the Shoreline Cleanup Assessment Technique (SCAT) established by the National Oceanic and Atmospheric Administration (NOAA, 2013; Santner et al., 2011). The SCAT Program collected sediment samples before the marshes were oiled in 2010. Five degrees of oiling were assigned based on visual assessments by NOAA teams: heavy, moderate, light, very light, and no oil observed (Michel et al., 2013). We used the initial SCAT maps from 03 August 2010 to identify one site with heavy oiling (hereafter “oiled”), and another site with no oil observed (“unoiled”), both within Barataria Bay near Port Sulphur, LA. We collected birds from each of these sites in August of 2011. Our collections in 2012–2014 were expanded to include birds from two additional sites identified as heavily
oiled in the SCAT surveys, as well as two additional sites known to have degrees of ‘very light’ or ‘no oil observed.’ There was a potential for storm systems to redistribute oil across the sites originally identified as oiled and unoiled in Barataria Bay, and so, beginning in 2013 we also collected birds from 2 to 3 additional unoiled sites from a different network of bays and channels on the north/east side of the Mississippi River near Pointe-a-la-Hache, LA. This second group of birds served as a reference (Table 1) that allowed us to examine if the enhanced CYP1A expression observed at our oiled and unoiled sites in 2013 represented a localized pattern (perhaps due to locally re-suspended oil within Barataria Bay), or was more widespread geographically.

2.2. CYP1A5 gene expression

Birds sampled in August 2011 were collected using a shotgun with 0.22 shotshells. From 2012 to 2014 birds were collected in June, following mist net capture within 50 m of the shoreline and were held in cloth bags until euthanization by thoracic compression. Protocols were approved by the Institutional Animal Care and Use Committee of the Louisiana State University AgCenter (permit A2012-05). Livers and other tissues were excised and immediately flash frozen in liquid nitrogen in the field, and later transferred to a −80 °C freezer where they remained until RNA extraction. A total of 116 birds were collected over the four years. Sampling effort focused primarily on adults (n = 103) but included some juvenile birds (n = 13). Birds were collected with the aim of keeping the sex ratio as even as possible (n = 63 male; n = 49 female; n = 4 unknown).

Livers were subsampled immediately upon thawing and a portion was homogenized in Buffer RLT Plus (Qiagen, Hilden, Germany) with a Tissue Tearor (Biospec, Bartlesville, OK, USA). The total RNA was then extracted from up to 600 μl of the resulting lysate (corresponding to no >30 mg tissue) using a Qiagen RNeasy Plus Mini Kit, and following the manufacturer’s instructions. The RNA concentration of each sample was determined by measuring the absorbance at 260 nm with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Subsets of RNA samples were run on agarose gels or examined with an Agilent 2011 Bioanalyzer (Santa Clara, CA, USA) to confirm RNA integrity. 1 μg of each RNA sample was treated with DNase and then reverse transcribed to cDNA using oligo dT primers, RNase inhibitor, and Superscript III reverse transcriptase (Invitrogen; Carlsbad, CA, USA).

Gene-specific primers were designed with Primer-BLAST (Ye et al., 2012) to amplify CYP1A5 which, along with CYP1A4, represents one of the two avian CYP1A genes. These genes share high sequence similarity resulting from a duplication event and similar patterns of induction.

Table 1

<table>
<thead>
<tr>
<th>Number of birds collected each year</th>
<th>2011</th>
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<td>5</td>
<td>16</td>
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<td>14</td>
</tr>
<tr>
<td>Reference</td>
<td>–</td>
<td>–</td>
<td>12</td>
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(Farmahin et al., 2013; Goldstone and Stegeman, 2006; Jonsson et al., 2011). We also designed primers to quantify expression of three candidate songbird reference genes (Zinow-Kramer et al., 2014): glyceraldehyde-3-phosphate dehydrogenase (GAPDH), peptidylprolyl isomerase A transcript variant 2 (PPIA), and ribosomal protein L4 (RPL4). PPIA and RPL4 were ranked as the most stable using a geNorm algorithm (Vandesompele et al., 2002); thus we used only these two reference genes to normalize our analyses. Primers were designed based on published avian sequences (Supporting Information S1).

Quantitative PCR reactions (10 μl) were run in three replicate reactions on Applied Biosystems 7900 Sequence Detection System instruments (Foster City, CA, USA) using PerfeCTa SYBR Green SuperMix, ROX (Quanta BioSciences, Gaithersburg, MD, USA) and primers at a concentration of 3 μM. Standard curves of 1:4 dilutions of cDNA were run to confirm acceptable reaction efficiencies of each primer set (CYP1A5, 107.9%; PPIA, 99.0%; RPL4, 102.1%). Reactions included 3 μl of cDNA that had been diluted 1:40. An initial denaturation at 95 °C for 3 min was followed by 40 cycles of 95 °C for 15 s, and then 60 °C for 45 s. A final dissociation stage of 95 °C for 15 s, 50 °C for 15 s, and 95 °C for 15 s was run after amplification to confirm single-product specificity of each sample.

A normalization factor based on the expression of the PPIA and RPL4 reference genes was used to normalize CYP1A5 expression (Vandesompele et al., 2002). We ran an arbitrarily chosen cDNA sample from each plate to serve as a calibrator, and samples from different years and sites were randomized across all qPCR runs. The Comparative Ct method (Livak and Schmittgen, 2001) was performed using DataAssist™ software to report the abundance of transcript for CYP1A5 relative to the calibrator, while controlling for the abundance of reference genes.

The Relative Quantity values were log2-transformed to satisfy assumptions of normality. All statistical analyses were performed using JMP Pro (Version 13.0, SAS Institute Inc., Cary, NC, 2011) for Microsoft Windows. A two-tailed independent samples t-test was first used to ask whether the hepatic CYP1A5 expression differed between birds sampled on a site that was heavily oiled following the spill and those sampled on a site that was initially unoiled. A factorial ANOVA was then used to explore the effects of year and treatment (degree of initial oiling of capture site: oiled or unoiled) on CYP1A5 expression in birds sampled in Barataria Bay each year from 2011 to 2014. A separate ANOVA examined hepatic CYP1A5 gene expression data from 2013 and 2014 only, which included data from birds collected at reference sites during those two years as a third ‘treatment’.

2.3. Sediment samples

The sediment samples described here were collected by us and other Louisiana State University (LSU) research groups within the Gulf of Mexico Research Initiative (GoMRI). Because these samples were acquired from several research groups with different sampling schedules, the sediment data points represent samples collected opportunistically across each year. For this reason, sample sizes and dates of collection varied within and among years and treatment types. A total of 194 sediment samples were collected in: 2011 (n = 27), 2012 (n = 31), 2013 (n = 68), and 2014 (n = 68), from oiled, unoiled, and reference sites (the latter in 2014 only). To maximize samples sizes, we included samples within 150 m of our bird sampling sites, provided the samples did not overlap with another site or SCAT category.

Sediment samples were collected in the 0 to 5 cm surface layer and within ~10 m of the shoreline. Gas chromatography/mass spectrometry in selective ion monitoring mode (GC/MS-SIM) on an Agilent 7890A GC system was used to determine the concentration of total alkanes and aromatic hydrocarbons (analysis by B. Meyer and E.B. Overton, LSU College of the Coast and Environment; analytical details are in Turner et al., 2014b).

Our data analyses focused on aromatic hydrocarbons because of the direct relationship between PAH exposure and CYP1A5 activity (Bowen et al., 2007). The concentration of total aromatic hydrocarbons in sediment samples were log-transformed prior to using a generalized linear model (GLM) to assess the effects of treatment (oiled or unoiled) and year (2011–2014) on aromatic concentrations. A second GLM was employed to investigate relationships among hepatic CYP1A5 gene expression, sediment aromatics, year, and treatment effects. The reference site data were not included in the analyses because samples were only available from 2014, although data from reference sites are shown in figures for comparison.

On August 28th, 2012, Hurricane Isaac made landfall near our study sites. To investigate the potential effects of this storm, 2012 samples were split into pre- and post-hurricane groups. Because seaside sparrow samples collected in 2012 were sampled in June, i.e. pre-Hurricane Isaac, they would not reflect any storm effects for that year. Therefore, to capture the aromatic concentrations for the period after the storm but before the next seaside sparrow collection in June 2013, post-hurricane soil samples taken in 2012 were pooled with the 2013 sediment samples in the analyses. The GLM was repeated after this regrouping of samples. This redistribution of samples resulted in a small sample size for the unoiled 2012 pre-hurricane treatment group (n = 2). Consequently, only oiled samples from the 2012 pre-hurricane and 2012 post-hurricane + 2013 groups were included in the two-tailed t-test used to determine if Hurricane Isaac influenced aromatic concentrations.

3. Results

3.1. CYP1A5 gene expression

The highest CYP1A5 expression observed in all samples were from birds collected in 2011 from oiled study sites (Fig. 2). The average expression level was about five times higher in these birds than it was in birds collected at the same time from unoiled sites (t25 = 3.26, p = 0.011), and at least twice as high as samples from any other year x treatment combination. A comparison of gene expression in oiled and unoiled sites for all years revealed a strong year x treatment interaction (F3,84 = 5.94, p = 0.001) because of the treatment effect in 2011. In following years, however, the treatment effect between oiled and unoiled sites disappeared.

The results of a factorial ANOVA also revealed a year effect that was independent of the treatment effect (F1,84 = 11.84, p < 0.001, Fig. 2). CYP1A5 expression was elevated in 2013 relative to 2012 and 2014 on both unoiled and oiled sites (Tukey HSD p < 0.001). Expression levels in 2014 for birds from oiled and unoiled sites returned to levels indistinguishable from those collected in 2012 (Tukey HSD p = 0.636).

The CYP1A5 response in 2013 was not restricted to birds collected in Barataria Bay: birds from reference sites showed nearly identical levels of CYP1A5 expression as those sampled from oiled and unoiled study sites in both 2013 and 2014 (Fig. 2). A comparison of the three treatments (oiled, unoiled, and reference) and the two years following Hurricane Isaac (2013 and 2014) revealed a strong year effect (F1,68 = 36.34, p < 0.001), but no treatment effect (F2,68 = 0.216, p = 0.807) or year x treatment interaction (F2,68 = 0.307, p = 0.737).

3.2. Sediment samples

Sediment samples demonstrated changes in total aromatic hydrocarbon concentrations across 2011–2014 at oiled and unoiled sites (Fig. 3). Concentrations ranged from 5.36 ng g⁻¹ (unoiled site) to 88,294.56 ng g⁻¹ (oiled site). The patterns in the mean PAH concentrations were similar to the CYP1A5 response data. Oiled samples sampled in 2011 had the highest mean PAH concentrations (9525.81 ng g⁻¹ ± 4499.13) as well as the greatest mean difference between concentrations at oiled and unoiled sites, however this difference was not significant in a two-tailed t-test (t35 = 1.5, p = 0.146).
A preliminary GLM, where samples were analyzed according to their original calendar year of collection, demonstrated treatment ($F_{1,178} = 3.85, p = 0.05$) and year ($F_{3,178} = 11.61, p = 0.009$) effects in aromatic concentrations, which distinguished 2012 from all other years. This result indicated that PAH concentrations were higher in samples collected in 2012 than in 2011. A two-tailed $t$-test used to assess whether the hurricane in 2012 influenced aromatic concentrations indicated significantly higher concentrations in samples collected from oiled sites after the storm ($t_{73} = 2.28, p = 0.026$) (Fig. 3). After grouping 2012 samples according to pre- and post-hurricane dates, the GLM showed a year ($F_{3,171} = 8.75, p = 0.033$) and year × treatment interaction effect ($F_{3,171} = 13.89, p = 0.003$). These results, however, were heavily influenced by the data from the unoiled 2012 pre-hurricane group, which consisted of only two samples. For this reason, we repeated the GLM without the 2012 pre-hurricane samples, revealing a strong treatment effect for the remaining years (2011, 2012 post-hurricane + 2013, 2014) (Table 2). Aromatic concentrations were significantly higher at oiled sites relative to unoiled sites ($F_{1,155} = 16.51, p < 0.001$). On average, oiled sites had more than eight times the aromatic concentrations than unoiled sites, as well as greater heterogeneity among samples (Fig. 3).

No relationship was found between CYP1A5 expression and aromatic concentrations ($F_{1,53} = 0.0886, p = 0.766$) in a GLM used to examine CYP1A5 expression as a function of sediment aromatics, year, treatment, and year × treatment, although it did confirm the findings of the earlier ANOVA that CYP1A5 expression varied by year ($F_{2,53} = 34.09, p < 0.001$), treatment ($F_{1,53} = 8.066, p = 0.005$), and year × treatment effects ($F_{2,53} = 16.57, p < 0.001$) (Fig. 3).

### 4. Discussion

Seaside sparrows showed significantly greater CYP1A5 induction on oiled sites within one year after the DWH spill, indicating a biological response to PAH exposure. Consistent with this finding, Bonisoli Alquati et al. (2016) showed lower radiocarbon ($^{14}$C) and stable carbon ($^{13}$C) values in the gizzard contents and feathers of seaside sparrows collected in 2011 from oiled sites, indicating oil incorporation. Our results are also generally consistent with changes observed in gene activity and other
Comparison, a method which accounts for likely oil biomarker weathering patterns (Meyer et al., 2018); the remaining 30% contained oil but were too weathered to be fingerprinted (S2). Furthermore, high wind and storm surge from Hurricane Isaac appear to have re-suspended and transported sediments from the continental shelf to the deep sea (Ziervogel et al., 2015), as well as depositing tar balls and tar mat fragments along the Alabama coastline (Clement et al., 2012). Re-suspension of oil that was initially deposited and buried in a patchwork of marsh areas in Barataria Bay or elsewhere across the Gulf Coast seems plausible (Brown and Brennan, 2012; Paruk et al., 2016), and the resulting redistribution would certainly be able to contribute to the widespread increase in CYP1A5 expression we observed after Hurricane Isaac. The rise of aromatics in 2012 post-Isaac + 2013 sediment samples supports this pattern and is similar to the results of Turner et al. (2014b) who noted a 6-fold increase in aromatics after the storm. Our ongoing research on marsh rice rats (Oryzomys palustris) from the same sites is expected to help clarify the extent to which such patterns generalize to other terrestrial marsh shore vertebrates potentially affected by the DWH.

Additionally, reduced abundances of above-ground arthropod species (e.g. not in direct contact with the substrate) in these marshes was noted in the aftermath of Hurricane Isaac (Bam, 2015). This may help to explain the apparent shift in dietary resources of seaside sparrows from a predominantly terrestrial trophic web, to an aquatic/benthic trophic web in 2013 relative to other years (Olin et al., 2017). If PAH content differed between prey items from terrestrial and aquatic/benthic food webs, then the differential CYP1A5 expression may be attributable to this shift in resource use. Considering the ubiquity of PAHs in the environment from both natural and anthropogenic sources, if seaside sparrows changed their diet to include more contaminated prey (or if spending more time foraging on the ground also entails more time preening off sediments the birds encounter) because a hurricane eliminated the bulk of their usual prey, then CYP1A5 expression could increase regardless of oil re-suspension. In either case, it’s possible that a dietary shift was compounded by the re-suspension of oil, elevating seaside Sparrow oil exposure after the storm. Based on the sediment sample data presented here, it seems likely that an increase in environmental PAHs heightened seaside Sparrow exposure. We expect that our ongoing studies of seaside Sparrow diet will help to clarify the source of contamination by identifying ratios of terrestrial and aquatically/benthically derived prey.

Ultimately, oil exposure, as revealed by CYP1A5 gene expression data, can have negative consequences for wildlife fitness, manifesting, for example, as changes to reproductive performance or success (Baos et al., 2012; Bergeon Burns et al., 2014) and differential survival (Esler et al., 2000). Our preliminary observations indicated reduced seaside Sparrow reproductive success on oiled sites. In 2012, 7% of nests on oiled sites (n = 28) successfully fledged versus 23% (n = 39) of nests on unoiled sites. The following year the success rate of oiled site nests was 5% (n = 19) and unoiled site nests was approximately 47% (n = 19) (Bergeon Burns et al., 2014). This is an outcome that will likely affect demographic parameters (although directly linking such effects to PAH exposure has not been possible). Fitness effects in other bird species after oil spills have been more subtle. For instance, following the Prestige oil spill in Galicia, Spain, the size of the red spot on the bill (a sexual signal) in yellow-legged gulls (Larus michahellis) was positively correlated to body condition and negatively correlated to an enzyme (aspartate aminotransferase) associated with liver damage in birds (Perez et al., 2010). Reproductive performance in female white stork (Ciconia ciconia) exposed to a mine spill was higher in an individual’s early years, but reproductive senescence also occurred earlier compared to unexposed birds (Baos et al., 2012). Up to nine years after the Exxon Valdez, differential survival of female harlequin ducks in oiled areas relative to non-oiled areas likely explained observed population declines (Esler et al., 2000). Other documented effects of PAH contamination in marine and freshwater birds, mammals, reptiles and amphibians

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**Table 2**

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
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include hemolytic anemia, immunosuppression, edema and developmental effects (Albers and Loughlin, 2003; Malcolm and Shore, 2003). Post-DWH effects in other terrestrial vertebrate species (excluding seaside sparrows) are less conclusive and to date are limited to the diamondback terrapin (Malaclemys terrapin) (Drabeck et al., 2014) and peregrine falcon (Falco peregrinus tundrius) (Seegar et al., 2015), which both spend a portion of their time in marsh habitats. Unfortunately, a small sample size precluded statistical analysis of PAH levels in the blood of diamondback terrapins (Drabeck et al., 2014) and the link between PAHs found in peregrine falcon blood and the DWH was inconclusive (Seegar et al., 2015).

Our results build on previous studies with new, quantitative evidence that the seaside sparrow, a terrestrial species residing in intertidal habitats, responded to oil exposure after the DWH spill. Because of their life history as an intertidal species feeding on invertebrates, seaside sparrows may be especially susceptible to residual oil exposure, prolonging their time to recovery (Bodkin et al., 2012; Esler et al., 2017a, 2017b), and indeed the data presented here indicate chronic exposure over several years. After any initial “acute” effects from encountering fresh oil have disappeared, the risk of exposure continues for species foraging in intertidal sediments where buried oil may linger (Esler et al., 2017b). Although population genetic work indicates that the seaside sparrows affected by the DWH spill are part of a widespread and relatively stable Gulf Coast population (subspecies fisheri) that can probably sustain some degree of loss, populations along the coasts of southern Texas (subspecies senneti) and northern Florida (subspecies juncicola and peninsularis) are smaller and genetically distinct, making them inherently more vulnerable to future oil spills or other anthropogenic disturbances in those regions (Elderd and Nott, 2008; Nelson et al., 2000; Woltmann et al., 2014).

Finally, it is important to acknowledge that oil was not evenly distributed across the marsh (Kirmann et al., 2016). This patchiness makes it difficult to sample sediment data in a representative way, and any inconsistencies between changes in CYP1A5 and the environmental concentration of aromatics may simply be a result of this difficulty. In such cases, CYP1A5 expression may be a better indicator of oil contamination than sediment samples, given that seaside sparrows and other species may move over a larger area and consume a variety of exposed prey.

5. Conclusion

As an abundant year-round resident and ground forager, the seaside sparrow is an important bioindicator of oil effects in marsh food webs in the aftermath of the DWH oil spill. We found that expression of CYP1A5 was markedly higher in the livers of birds sampled in an area recovering from heavy oiling compared to unoiled areas in the first year after the spill. The significance of these data are strengthened by the high PAH levels in sediments, confirming that terrestrial species that use intertidal habitats warrant consideration for studies of effects after marine oil spills. After apparent recovery in 2012, CYP1A5 expression was elevated again in 2013, this time across all study sites, possibly because of the redistribution of previously deposited oil during Hurricane Isaac. A comprehensive understanding of the effects of the 2010 DWH spill should include the influence of subsequent weather events that release and redistribute oil throughout the landscape and food web. Continued monitoring of seaside sparrows may allow us to observe additional episodic effects over the long-term, associated with the frequent storms in this region.

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Appendix A. Supplementary data

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References


Bam, W., 2015. Effects of Oil Spill and Recovery of Terrestrial Arthropods in Louisiana Saltmarsh Ecosystem. (Master’s thesis). Louisiana State University, Baton Rouge, LA, USA.


Clement, T.P., Hayworth, J.S., Mulabagal, V., John, G.F., Yin, F., 2012. Research Brief-II: Impact of Hurricane Isaac on Mobilizing Deepwater Horizon Oil Spill Residues Along...


vanEwijk-Watson, S., et al., 2015. Adrenal gland and lung lesions in Gulf of Mexico common bottlenose dolphins (Tursiops truncatus) found dead following the Deepwater Horizon oil spill. PLoS ONE 10, e0126538.


