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Changes in the concentration and relative abundance of alkanes and PAHs from the Deepwater Horizon oiling of coastal marshes

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ABSTRACT

We determined changes of 28 alkanes and 43 different PAHs in 418 wetland soil samples collected on ten sampling trips to three Louisiana estuaries before and after they were oiled from the 2010 Deepwater Horizon disaster. There was a significant decline in 22 of the 28 alkane analytes (0.42% day^{-1}), no change in 6, over 2.5 years. The concentration of five aromatic petroleum hydrocarbons (PAHs) increased (range 0.25–0.70% day^{-1}), whereas the total PAH pool did not change. Of these five, naphthalene and C-1-naphthalenes are suggested to be of higher toxicity than the other three because of their relatively higher volatility or solubility. The relative proportions of alkane analytes, but not PAHs, does not yet resemble that in the pre-oiled marshes after 3 years, The trajectories of nine indicators for degradation/weathering were either inconclusive or misleading (alkanes) or confirmed the relatively meager degradation of PAHs.

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1. Introduction

1.1. Oiling the marsh

The BP Macondo Blowout disaster (also known as: DWH; Deepwater Horizon) began at Mississippi Canyon Block 252 (MC252) and released an estimated 4.9 million barrels of South Louisiana crude oil (MC252) into the Gulf of Mexico from 20 April to 15 July 2010 (Crone and Tolstoy, 2010). It is the largest unintended release of oil to the marine environment in US history and resulted in the death of 11 people and injured 17. The distribution of oil from the 'spill' was assessed under the Shoreline Cleanup Assessment Technique Program (SCAT) comprised of Federal, State, local and industry representatives. The results indicate that 1773 km of Gulf of Mexico shoreline were significantly oiled, of which 45% was coastal marsh. The majority of the heavily and moderately oiled areas were in Louisiana. Oil mitigation or clean-up occurred on 8.9% of the oiled marsh shoreline (Michel et al., 2013).

Depending on initial conditions of oiling and other factors, petroleum hydrocarbons can persist for years (Teal et al., 1992; Reddy et al., 2002) and with sustained biological effects (Culbertson et al., 2007a,b). The average concentration of total alkanes and polycyclic aromatic hydrocarbons (PAHs) in these marshes, in June 2013 were 20× and 374× the pre-oiled conditions (Turner et al., in preparation). The concentration of total alkanes are on a trajectory to be near baseline levels by 2015, whereas the concentration of PAHs may take many decades to reach baseline levels if no additional oiling occurs. The concentration of PAHs in June 2013 was at levels that affect the reproduction and growth of resident fish (Whitehead et al., 2012; Turner et al., in preparation). Shoreline erosion was accelerated (McClenachan et al., 2013) and insect communities were depressed (Pennings et al., 2014).

1.2. This study

This analysis is concerned with changes in the concentration of the individual alkane and PAH analytes that make up the total alkane and PAH pool measured in these marshes. We examined data from before the arrival of MC252 oil to three years after it arrived. Our main objectives were to examine: (1) the changing composition of the oily residues in coastal marshes and (2) evidence for biodegradation/weathering using nine indices of change.

1.3. General aspects of change

The analysis of petroleum hydrocarbons typically divides compounds into two chemical groups that will have different trajectories of change in the marsh. The alkanes are saturated

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hydrocarbons with single bonds for the carbon-to-carbon linked chains. They are neither very reactive nor identified as major health hazards. Degradation is principally by microbial oxidation of the terminal carbon atom. PAHs, in contrast, form six-carbon rings of alternating single and double carbon atoms and are potent pollutants. Some PAH compounds are carcinogenic, mutagenic, or teratogenic (Luch, 2005).

A variety of factors will affect the degradation rate of various alkanes and PAHs of the MC252 oil in the marsh. Some major factors are the molecular weight or carbon number (C_n), volatility, solubility, eH (oxidation potential) and pH, sediment organic content, sediment type, oxygen availability, and temperature. Alkanes with a lower molecular weight are more easily volatilized, whereas the mid-length (C_n = 14–20) alkanes are generally non-polar liquids (the melting point of C20 is 37 °C), with very minimal water solubilities, whose melting and boiling points increase as their carbon numbers increase. Aromatics are 100 times more soluble in water than alkanes of the same carbon number (McAuliffe, 1966). This means that alkanes may be more prone to evaporative losses than are the aromatics. Hydrocarbons have been generally ranked in the following order of decreasing susceptibility to microbial metabolism: n-alkanes < branched alkanes < low molecular weight aromatics < cyclic alkanes < high molecular weight aromatics $\lll$ hopanes and steranes hydrocarbons (Leahy and Colwell, 1990). The evaporative losses of MC252 oil moving from the wellhead to shore appears to be more important than microbial weathering of the surface oil because 64% of the MC252 source oil PAH pool were the easily volatilized 10 and 12-carbon naphthalenes (Liu et al., 2012). In general, the higher the molecular weight of PAHs, the higher the hydrophobicity and toxicity, and the slower the compound is to degrade. Collectively, these physicochemical properties mean that mid-length aromatic hydrocarbons are not readily volatilized or leached from soil and can remain toxic unless degraded to non-toxic forms.

2. Methods

2.1. Sample collection

We collected 418 wetland soil samples on ten sampling trips in three coastal Louisiana estuaries. Their locations and distributions are described in Turner et al. (in preparation). The first collection was in May 2010 (n = 31) in the Breton Sound, Barataria and Terrebonne estuaries before the oil from the Macondo well blowout entered the wetlands. Nine additional sampling trips were in September 2010 (n = 64) and February 2011 (n = 30), May 2011 (n = 87), September 2011 (n = 66), June 2012 (n = 22), August 2012 (n = 30), September 2012 (n = 30), October 2012 (n = 28), and June 2013 (n = 30). The February 2011, August 2012, September 2012, and June 2013 sampling trips went to the same 30 individual sites and are from within 2 × 2 m plots. Soil samples were collected as a composite sample of the upper 5 cm and stored on ice until delivery to the laboratory, and either immediately extracted or refrigerated for no more than 14 days at 4 °C until extraction, as recommended by the US EPA (2007). MC252 source oil collected from the riser structure at the accident site and surrendered to the US Coast Guard was extracted and analyzed as described for all field samples.

2.2. Sample analysis

Sample analysis is described in detail in Turner et al. (in preparation), which discusses changes in the total alkanes and total PAHs in time and space. That study identified 28 alkanes and 43 aromatic hydrocarbons and their respective alkyl homologs (18 parent PAHs, and 25 alkyl homolog groups) using GC/MS-SIM (gas chromatography/mass spectrometry in selective ion monitoring mode), including the normal and branched saturated hydrocarbons (from C10 to C35), the one- to five-ringed aromatic hydrocarbons and their C1–C4 alkyl homologs, as well as cyclic biomarker compounds like the hopanes, steranes and triaromatic steroids (SIM ions 191, 217, 218, and 231 eluting between C_{23} and C_{31}).

Briefly, the samples were analyzed using accepted standard operating and QA/QC procedures to prevent contamination and avoid sample degradation. The soils were extracted with dichloromethane (DCM) and spiked with surrogate standards to achieve a final extract concentration of 20 μg mL^{-1}. The concentrations of the target PAHs were determined by an internal standard method and response factors calculated from a 5-point calibration curve using a commercially-available standard containing the normal alkanes from n-C10 through n-C35 and the parent PAH analytes of interest. The MS detector was tuned to PFTBA (perfluorotributylamine) before each set of analyses. If any of the tune parameters (e.g., percent air/water, peak abundances and ratios) were significantly different from before tune parameter values, then the instrument was checked for errors and then returned to normal operating conditions. A daily calibration standard and blank were analyzed with each sample batch to verify proper instrument performance. If the daily standard indicated instrumental problems, then no further analytical work was performed until the instrument was restored to good operating condition.

The identities of all analytes were established using retention times and full scanning mass spectral data of the riser oil sample. The spectral data were processed by Chemstation Software (Agilent Technologies). The data are archived at https://data.gulfresearchinitiative.org/data/R1.x139.142:0004/.

2.3. Data analysis

Changes, both higher and lower (% day^{-1}), for the various alkane analytes were calculated using the slope of natural log of the averages vs. day, from the eight sampling trips taken from September 2010 to October 2012. Decay rates could not be calculated when the concentration was below the detection concentration. We did not use the June 2013 data for this part of the analysis because the concentration of some alkane analytes and the total alkane pool was near the baseline value by October 2012.

Nine indices of oil weathering were calculated: (A) n-heptadecane: pristane versus year; (B) n-octadecane: phytane versus year; (C) pristane: phytane versus year; (D) C3-dibenzothiophenes: C3-phenanthrenes versus year; (E) C3-dibenzothiophenes: C3-chrysenes versus year; (F) n-heptadecane/n-pentatricontane versus years; (G) n-heptadecane versus pristane; (H) n-octadecane versus phytane; (I) pristane versus phytane. The proportional amount of individual alkane or aromatic analytes were also calculated as the percent of the total amount of measured alkanes or PAHs. All reported statistical analyses are significant at $p \leqslant 0.05$.

3. Results

3.1. Relative concentrations in alkanes and PAHs

A comparison of alkane and PAH analyte concentrations just before the MC252 oil arrived in the marshes (May 2010), just after it arrived (September 2010), and three years later (June 2013) is in Fig. 1. The average concentration of total alkanes and total PAHs in September 2010 was 134× and 110×, respectively, the pre-oiled conditions. The concentration of total alkanes declined to 20× that of the pre-oiling conditions by October 2012. The concentration of total aromatics in October 2012 was 374 times higher than

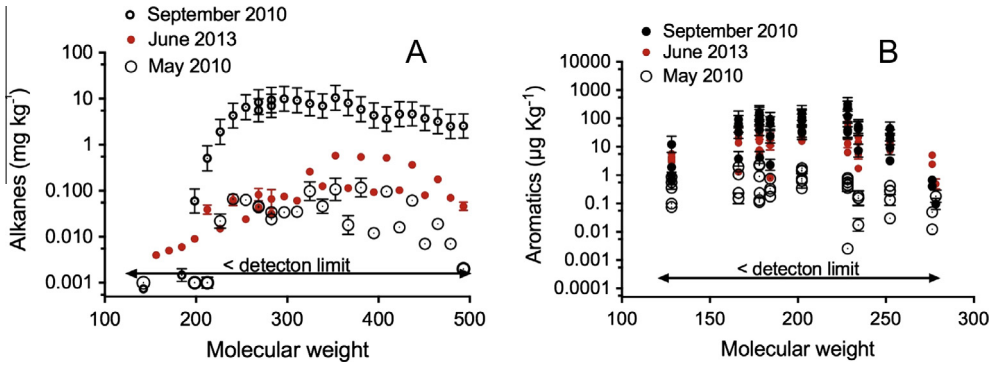

Fig. 1. The concentration ($\mu \pm 1$ SE) of alkanes (A) and aromatics (B) in May 2010 ($n = 31$), September 2010 ($n = 64$), and June 2013 ($n = 30$) (Y axis) and the molecular weight (x axis). The aromatics have multiple analytes with the same molecular weight, but different molecular structures. Analytical results below the detection limit are indicated for visual illustration. The detection limits vary for each analyte.

pre-oiling conditions – which suggests that significant re-oiling of the marsh occurred. There was a significant decline with time among all trips for the total alkane concentration (0.39% day^{-1}; $p = 0.05$), but not for the total PAHs (Turner et al., in preparation).

3.1.1. Alkane analytes

There were no alkanes detected among the analytes examined that had a molecular weight <190 (n-decane, n-undecane, n-dodecane, and n-tridecane) in May and September, 2010, and only sparse amounts in June 2013 (Fig. 1A). There was a steady rise in the concentration of alkanes as molecular weight increased to 250 in the first months of oiling (September 2010), and then a decline. This pattern of concentration versus molecular weight was sustained through all sample dates, up to June 2013 (Fig. 1A). The alkane analyte concentrations in September 2010 were higher compared to June 2013, except for the three lowest molecular weight compounds (n-decane, n-undecane, and n-dodecane) that were undetectable in May 2010.

The decay rates (% day^{-1}) of the 28 alkane analytes for the eight data sets collected from September 2010 to October 2012 are in Fig. 2, arranged according to their molecular weight. There was no trend in the concentration of the relatively lighter alkane analytes for n-decane, n-dodecane, n-tridecane, n-tetradecane, n-pentadecane, n-hexadecane, n-heptadecane, pristane or phytane, ranging from 142 to 212 molecular weight. The average value for the 22 with statistically-significant declines in concentration was −0.40% day^{-1}, which is similar to the −0.39% day^{-1} rate observed for the total alkane pool ($C_n = 17$–33).

3.1.2. Aromatic analytes

There were occasionally samples with undetectable concentrations of aromatics in May 2010 (before oiling). The average concentration of all aromatic analytes were higher three years after oiling (Fig. 1B).

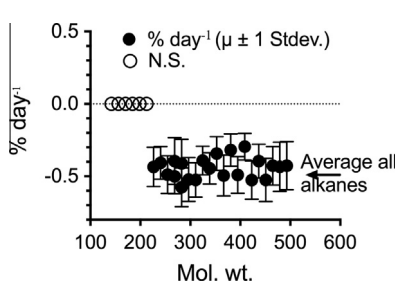

Fig. 2. The decay rates (% day^{-1}) of alkanes from September 2010 to October 2012. Unfilled circles represent statistically-insignificant changes ($p > 0.05$). The unfilled circles had no detectable change in concentration.

An increase in concentration over the sampling trips from September 2010 to September 2012 was measured for naphthalene, C-1-naphthalenes, benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene, which ranged between 0.25% and 0.70% day^{-1} (Fig. 3). The change in concentration for the total PAHs for the eight post-oiling sampling trips was a statistically insignificant −0.17% day^{-1}.

3.2. Relative change in analytes

The proportional amount of alkane analytes and PAH analytes in the oily residues increased with increasing molecular weight (Fig. 4A–F). The proportional amount of alkane analytes in the MC252 source oil found in the marsh had relatively small amounts of the lighter weight alkanes (100–200 MW) or PAHs in September 2010 (Fig. 4B and E). The proportional amount of alkanes in September 2010 (Fig. 4B), and June 2013 (Fig. 4C) is starting to match what was in the marsh before MC252 oil arrived in the summer and fall 2010, but the total amount, of course, was much higher.

The proportional amounts of PAHs for May 2010, September 2010, and October 2012 (Fig. 4D–F), however, are quite different from that of the alkanes (Fig. 4, right panel). The relative proportions in May 2010 had four aromatic analytes above 10% of the total, and four others between 2% and 10% of the total. The comparable distribution in September 2010 is that there were 1 and 6 analytes above 10%, and between 2% and 10%, respectively, whereas three years later the distribution was 1 and 14 above 10%, or between 2% and 10%, respectively. There were 2 aromatic analytes above 2% of the total aromatics in May 2010, none in September 2010, and eight in October 2012.

3.3. Increases in analytes

The concentration of naphthalene and C1-naphthalene increased disproportionately to the amount of oiling. The relative amounts of naphthalene and C1-naphthalene in May 2010 were 2.2% and 0.4%, respectively, of the total aromatic pool. The proportional amount of naphthalene and C1-naphthalene were 0.07% and 0.02%, respectively, of the total aromatics in September 2010, 0.15% and 0.2%, respectively, by October 2012, and 1.2% and 0.5% by June 2013. The average concentration of naphthalene and C1-naphthalene in October 2012 (45.2 ± 43.2 and 7.0 ± 17.2 ng g^{-1}, respectively) was equal to 24× and 17× times the average amount present in September 2010. In June 2013 the average concentration of naphthalene and C1-naphthalene had fallen to 2.36 ± 1.87 and 0.93 ± 2.10 ng g^{-1}, respectively.

The concentration of the three heavier aromatics that increased (benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene)

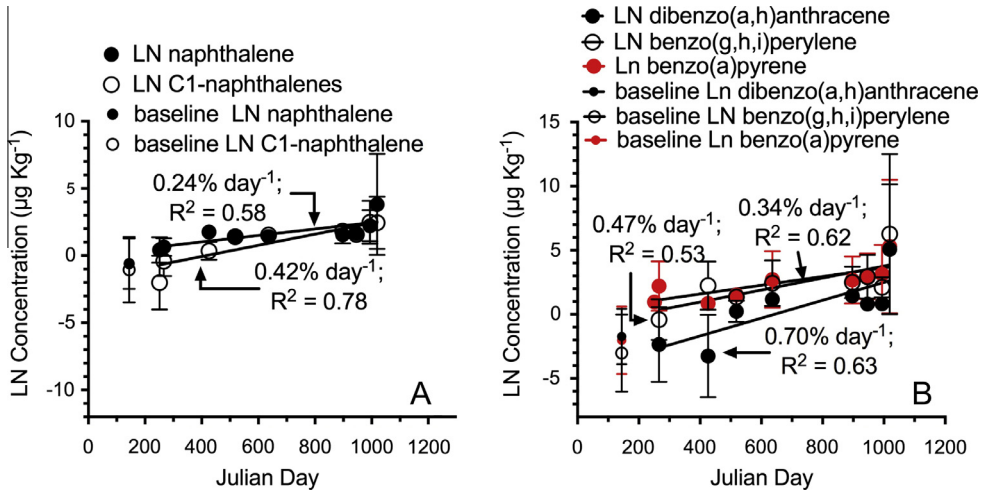

Fig. 3. The natural log concentration ($\mu \pm 1$ SE) of aromatics versus Julian Day (beginning 1 January 2010): (A) napthalene and C-1-naphthalenes; and, (B) benzo(a)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene. The baseline values are for May 2010 when MC252 oil had not yet made landfall in the marsh. A linear fit of the data is shown ($p < 0.05$).

went from 0.13, 0.18, and 0.05 ng g^{-1}, respectively, before the marshes were oiled to 9.13, 0.10, and 0.66 ng g^{-1}, respectively, in September 2010. By October 2012, the comparable concentrations were 61, 1656 and 784 ng g^{-1}, equivalent to 61×, 1656× and 784× higher, respectively, than in September 2010.

3.4. Indicators

All nine indicators of alkane and aromatic analyte degradation failed to suggest differential rates of weathering for different alkane analytes (Fig. 5), even though there was a loss of total alkanes over the two years. The losses of individual alkanes between C-17 and C-33 was similar (Fig. 2), and so the ratios showed no changes over time, or when compared with each other. The absence of a clear trend in the two indices of aromatic degradation is consistent with the idea that the total PAH concentration in the sediments was relatively stable compared to the alkanes.

4. Discussion

4.1. Persistence

The decomposition of organics in anaerobic soils is, in general, considerably slower than in aerobic soils. These coastal Louisiana marsh soils are anaerobic and organic-rich. One to 10 m beneath them are the silts, clays and sand from the Mississippi River. Above these inorganics is a surface layer comprised mostly of organic matter accumulating from the centuries of plant growth, especially of roots and rhizomes. It is the accumulation of this organic material that keeps the marsh at equilibrium with sea level changes (Turner et al., 2000). The anaerobic soil conditions in these soils facilitate minimal oil degradation (Hambrick et al., 1980; Delaune et al., 1981). Laboratory studies by Bauer and Capone (1985), for example, revealed no remineralization of either anthracene or naphthalene intertidal soils without oxygen, and that the process was temperature dependent. The results of other laboratory studies by Boyd et al. (2005) revealed that the oxygen supply was the determining factor controlling PAH degradation. This slow degradation is not uncommon for salt marsh soils. Reddy et al. (2002) found that pristane, phytane and other branched alkanes were still present 30 years after the West Falmouth oil spill (1969, MA) and predicted that "hydrocarbons contamination will persist indefinitely in the sedimentary record".

These results suggest that the persistence of the oil in the marsh (from the original oiling and probable re-oiling) may be a continuing stressor, in combination with other stressors like tropical storms and nutrient pollution, on the marsh ecosystem. Indeed, enhanced marsh erosion has been noted by Peacock et al. (2007) analysis of the Bouchard 65 oil barge release of No. 2 fuel oil in 1974, and soil toxicity (to amphipods) remained 7 years after the Chalk Point oil spill (Maryland; Michel et al., 2009). These two results also suggest that the legacy of PAHs is a continuing stressor on the emergent plants that have a cascading effect on the interdependent ecosystem structure and functions. Culbertson et al. (2007a) documented how the growth, condition index and filtration rate of the ribbed mussel (*Geukensia demissa*), which is co-located at the base of the dominant emergent salt marsh grass (*Spartina alterniflora*), were affected by remnants of oil deposited 38 years earlier.

Venosa et al. (1996) studied a sandy beach with aerated soils and found much faster decay rates (defined as '*K*' values) for the control plots (−2.6% and −2.1% day^{-1} for total alkanes and total aromatics, respectively) which is 8 times higher than the values we measured for individual alkanes. We also found that there was no decline in the aromatic analytes, however, and five instances of the concentration of analyte increasing. This contrast between the analyte declines in aerated and anaerobic soils is consistent with what other authors have found, except for the rise in some PAH analytes.

4.2. Indices of degradation

The degradation of alkanes might be expected to result in a decline in the C17: pristane ratio and the nC18: phytane ratios (Ward et al., 1980; Diaz et al., 2000; Oh et al., 2001; Pritchard and Costa, 1991; Qimin et al., 2007; Liu et al., 2012), and an increase in the pristane: phytane ratio (Diaz et al., 2000; Qimin et al., 2007). This is because the presumably less recalcitrant analytes (lower molecular weight) would degrade, solubilize, or evaporate at a different (faster) rate than others. The absence of change in the indicators examined in this study suggests a slow degradation rate. However, we appreciate that the hydrocarbons in these soils are a mixture of biogenic source material that is mixed with pre-Macondo spill oil and Macondo oil, which complicates interpretation of the fate of recently-deposited petrogenic hydrocarbons.

The lack of changes in the indices of degradation for alkanes is, perhaps, surprising in view of the decline in total alkanes of several

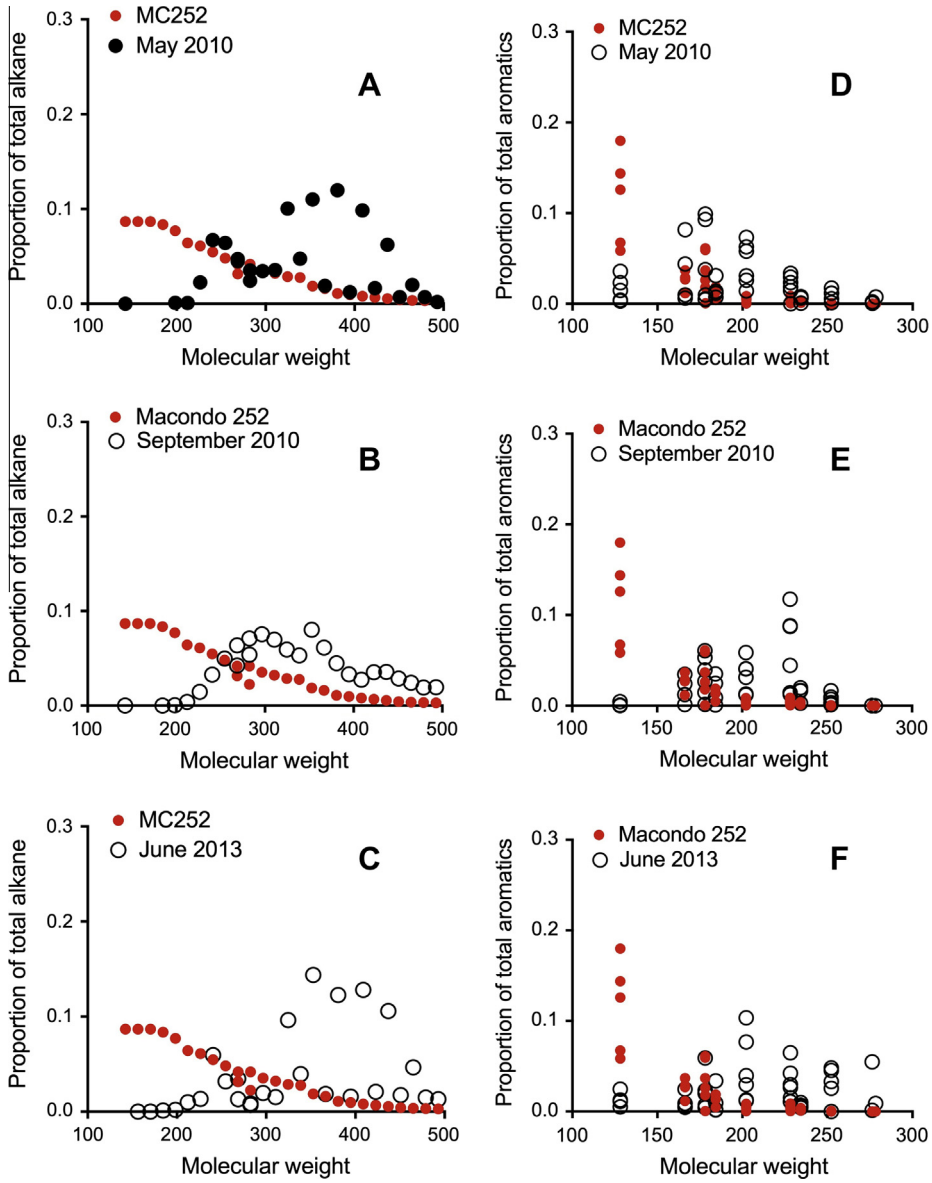

Fig. 4. The relative proportion of measured alkanes (A–C) and aromatics (D–F) in samples collected in May 2010 ($n = 31$), September 2010 ($n = 64$), and June 2013 ($n = 30$) versus the molecular weight (x axis). The red dots are for oil from the Macondo Deepwater Horizon wellhead.

orders of magnitude (Fig. 1). The ratios might not change in our samples, however, because the losses of individual alkanes between C-17 and C-33 were similar (Fig. 2), and so the ratios showed no changes over time, or when compared with each other. The decline in total alkanes was pervasive and somewhat homogeneous, and cannot be explained by dilution or burial because there was no concomitant decline in the total aromatics.

Douglas et al. (1996) suggested that the two- and three-ring aromatic compounds are more readily degraded than the four-ring chrysenes. If the latter occurs, then both the C3-dibenzothiophenes: C3-chrysene and C3-dibenzothiophenes: C3-phrenanthenes ratios would decline with time, and they do not (Fig. 5E and D). This pattern is in agreement with the stability of the total PAH concentration over time.

4.3. Net increases in PAH constituents

We did not expect to find the increase in some aromatics, but that possibility is obliquely suggested by Ward et al. (1980) who studied the concentration of various PAHs in salt marsh soils after the Amoco Cadiz spill. They made this brief remark at the very end of their paper: "The long term persistence of Amoco Cadiz hydrocarbons may be related to lower biodegradability of residual *or newly formed hydrocarbons*." (Italics added). They suggested, in other words, the potential of a net rise in some PAH molecules, although they did not document it. We know of no published report documenting a rise in a PAH analyte in anaerobic soils.

We found that there has been a measurable and large increase in the concentration of the relatively low molecular weight naphthalene and C-1-naphthalenes (10 times higher from September 2010 to October 2012), and in the much higher molecular weight analytes benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i) perylene (100–1000 times higher). The two lower weight molecules have a relatively high volatility and solubility. All five are considered to be toxic. Naphthalene, in particular, has been a known insecticide for decades (e.g., Tattersfield 1928).

The potential for the movement of oil and its by-products between sites is a potentially confounding factor interpreting changes in concentration in this analysis of 0–5 cm sediment samples. The visual impression of oiling on the shoreline in 2010 was

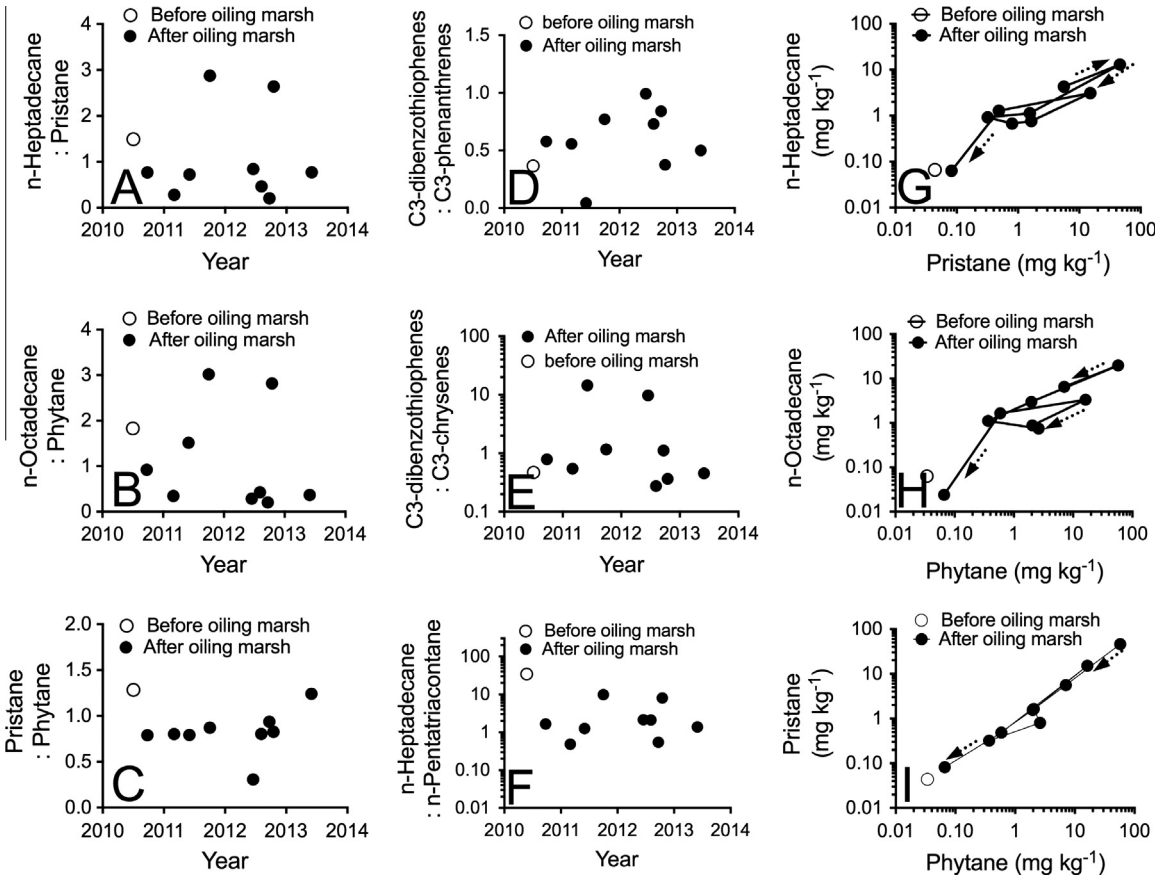

Fig. 5. Indices of oil degradation for alkanes (mg kg^{-1}) and aromatics (μg kg^{-1}): (A) n-heptadecane: pristane versus year; (B) n-octadecane: phytane versus year; (C) pristane: phytane versus year; (D) C3-dibenzothiophenes: C3-phrenanthrenes versus year; (E) C3-dibenzothiophenes: C3-chrysenes versus year; (F) n-heptadecane/n-pentatricontane versus years; (G) n-heptadecane versus pristane; (H) n-octadecane versus phytane; (I) pristane versus phytane. There was no detectable trend in the ratios with time (A–F). The line in G–I connects data from each sampling trip sequentially.

one of a heterogeneous distribution and it may have become more homogenous with flooding during storm surges. McClenachan et al. (2013), for example, measured oil concentrations that were 10 times higher at sites 10 m apart. Oil concentrations measured within thirty 1-m^2 quadrats were ten times higher one month after Hurricane Iassac compared to one month before (Turner et al., in preparation). In the larger view, though, the consistency of the declines in the total alkanes and PAHs and of most individual alkanes and aromatics, when compared to the rise in the concentration of only a few aromatics suggests an *in situ* source, and not one that is the result of lateral transport.

4.4. Distinguishing toxicity

Speculations on the relative toxicity of the five compounds with increased concentrations are plausibly related to what is known about how their molecular weight is related to its toxicity, volatilization, and solubility. Sverdrup et al. (2002) measured the toxicity of 16 PAH analytes to the soil-dwelling springtail *Folsomia fimetaria*, a common toxicity test animal. They found that the springtail was more sensitive to lower molecular weight analytes, including naphthalene, than the three high molecular weight analytes that increased in our analyses. They also quantified the indirect relationship between water solubility and molecular weight, meaning that lower molecular weights are much more soluble than heavier molecules. A similar relationship is observed between volatilization and molecular weight. A marsh releasing naphthalene to the atmosphere would, therefore, expose organisms at the soil–water interface (and above) in greater amounts in warm weather when water was off the marsh and during calm winds, and least in cooler temperatures when the marsh was covered with water, and experiencing strong winds.

But this speculation also occurs within the matrix of known interactive and cumulative effects of multiple toxins, of which relatively few are measured. The identified PAH and alkane pool normally investigated that are known to be important indicators of the oil residue's composition and weathering patterns, for example, are in the range of 5% using conventional analytical methods (this study), and less than 25% with more extensive analyses (Reddy et al., 2012). There may be other compounds degraded that create new toxic compounds, including synergistic effects.

It is not a speculation that some of the degradation products are as toxic as the parent compound. In vitro studies by Cerniglia (1984), for example, have shown the possible production of intermediates from PAH degradation, particularly dihydrodiols, which are of greater toxicity than the parent compound. Also, Lundstet et al. (2003) measured degradation products from a bioreactor treating PAH-contaminated gasworks soil. They monitored the accumulation of oxy-PAHs, such as PAH-ketones, quinines and coumarins that formed or increased concentration during the metabolism of the PAH. The addition of a fertilizer to enhance oil degradation resulted in 10 times more neutral oxy-metabolites of hydrocarbons that exhibit significantly higher toxicity to developing atherinid and clupeoid fish embryos (Middaugh et al., 1996, 1998) and Inland Silversides (*Menidia beryllina*; Middaugh et al., 2002). The damage to these fish included embryo malformations,

genetic damage, and mortality. We conclude that it would be a risky proposition to assume that the toxicity of some oil analytes have been in decline since the marshes were first oiled in 2010.

4.5. Conclusions

The organic rich and anaerobic salt marsh soils that were heavily oiled by the MC252 oil in summer 2010 are a significant reservoir of 28 alkanes whose concentrations declined to <10% of their peak, but remain 15 times higher than before the oiling as of October 2012. The lighter alkanes in the parent oil are largely absent, and are dominated by the alkanes with C_n between 14 and 33. The concentrations of 43 aromatics, in contrast, are fairly stable and five of them increased. Of these five, we speculate that the relatively light PAHs, naphthalene and C1-naphthalenes, are more likely to be of concern as being a toxic burden larger than when first oiled. The specific chemical source and pathway creating this additional toxic burden are unknown, and may be accompanied by unidentified degradation products from the unexplored 98% of the oil that is not routinely documented. The average concentration of PAHs remaining as of June 2013, equals or exceeds the level at which stressors occur in the common marsh fish, *Fundulus grandis*, plants, and insect community. At current trajectories of change, some hydrocarbons will remain for many more decades, as will the impacts, which remain subtle and largely unexplored.

Acknowledgments

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References

Bauer, J.E., Capone, D.G., 1985. Degradation and mineralization of the polycyclic aromatic hydrocarbons antracene and naphthalene in intertidal marine sediments. Appl. Environ. Microbiol. 50, 81–90.
Boyd, T.J., Montgomery, M.T., Steele, J.K., Pohlman, J.W., Reatherford, S.R., Spargo, B.J., Smith, D.C., 2005. Dissolved oxygen saturation controls PAH biodegradation in freshwater estuary sediment. Microbial Ecol. 49, 226–235.
Cerniglia, C.E., 1984. Microbial metabolism of polycyclic aromatic hydrocarbons. Adv. Appl. Microbiol. 30, 31–37.
Crone, T.J., Tolstoy, M., 2010. Magnitude of the 2010 Gulf of Mexico oil leak. Science 330, 634.
Culbertson, J.B., Valiela, I., Olsen, Y.S., Reddy, C.M., 2007a. Effect of field exposure to 38-year-old residual petroleum hydrocarbons on growth, condition index, and filtration rate of the ribbed mussel, *Geukensia demissa*. Environ. Pollut. 154, 312–319.
Culbertson, J.B., Valiela, I., Peacock, E.E., Reddy, C.M., Carter, A., Van der Kruik, R., 2007b. Long-term biological effects of petroleum residues on fiddler crabs in salt marshes. Mar. Pollut. Bull. 54, 955–962.
Delaune, R.D., Patrick Jr., W.H., Casselman, M.E., 1981. Effect of sediment pH and redox conditions on degradation of benzo(a)pyrene. Mar. Pollut. Bull. 12, 251–253.
Diaz, M.P., Grigson, S.J.W., Peppiatt, C.J., Burgess, J.G., 2000. Isolation and characterization of novel hydrocarbon-degrading euryhaline consortia from crude oil and mangrove sediments. Mar. Biotechnol. 2, 522–532.
Douglas, G.S., Bence, A.E., Prince, R.C., McMillen, S.J., Butler, E.L., 1996. Environmental stability of selected petroleum hydrocarbon source and weathering ratios. Environ. Sci. Technol. 30, 2332–2339.
Hambrick III, G.A., DeLaune, R.D., Patrick Jr., W.H., 1980. Effect of estuarine sediment pH and oxidation–reduction potential on microbial hydrocarbon degradation. Appl. Environ. Microbiol. 40, 365–369.
Leahy, J.G., Colwell, R.R., 1990. Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. 54, 305–315.
Liu, Z., Liu, J., Zhu, Q., Wu, W., 2012. The weathering of oil after the Deepwater Horizon oil spill: insights from the chemical composition of the oil from the sea surface, salt marshes and sediments. Environ. Res. Lett. 7. http://dx.doi.org/10.1088/1748-9326/7/3/035302.
Luch, A., 2005. The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons. Imperial College Press, London.
Lundstet, S., Haglund, P., Öberg, L., 2003. Degradation and formation of polycyclic aromatic compounds during bioslurry treatment of an acid aged gasworks soil. Environ. Toxicol. Chem. 22, 1413–1420.
McAuliffe, C., 1966. Solubility in water of paraffin, cycloparaffin, olefin, acetylene, cycloolefin, and aromatic hydrocarbons. J. Phys. Chem. 70 (4), 1267–1275.
McClenachan, G., Turner, R.E., Tweel, A.W., 2013. Effects of oil on the rate and trajectory of Louisiana marsh shoreline erosion. Environ. Res. Lett. 8, 044030.
Michel, J., Nixon, Z., Dahlin, J., Betenbaugh, D., White, M., Burton, D., Turley, S., 2009. Recovery of interior brackish marshes seven years after the chalk point oil spill. Mar. Pollut. Bull. 58, 995–1006.
Michel, J., Owens, E.H., Zengel, S., Graham, A., Nixon, Z., Allard, T., Holton, W., Reimer, P.D., Lamarche, A., Whit, M., Rutherford, N., Childs, C., Mauseth, G., Challenger, G., Taylor, E., 2013. Extent and degree of shoreline oiling: Deepwater Horizon oil spill, Gulf of Mexico, USA. Plos One 8, e65087.
Middaugh, D.P., Shelton, M.E., Chapman, P.J., 1996. Responses of embryonic and larval inland silversides, *Menidia beryllina*, to a water-soluble fraction formed during biodegradation of artificially weathered Alaska North Slope crude oil. Arch. Environ. Contam. Toxicol. 31, 410–419.
Middaugh, D.P., Shelton, M.W., McKenney, C.L., Cherr, G., Chapman, P.J., Courtney, L.A., 1998. Preliminary observations on responses of embryonic and larval Pacific Herring, *Clupea pallasi*, to neutral fraction biodegradation products of weathered Alaska North Slope oil. Arch. Environ. Contam. Toxicol. 42, 236–343.
Middaugh, D.P., Chapman, P.J., Shelton, M.W., McKenney, Courtney., 2002. Effects of fractions from bioegraded Alaska North slope crude oil on embryonic inland silversides, *Menida beryllina*. Arch. Environ. Contam. Toxicol. 34, 188–196.
Oh, Y.-S., Sim, D.-S., Kim, S.-J., 2001. Effects of nutrients on crude oil biodegradation in the upper intertidal zone. Mar. Pollut. Bull. 42, 1367–1372.
Peacock, E.E., Hampson, G.R., Nelson, R.K., Xu, L., Frysinger, G.S., et al., 2007. The 1974 spill of the Bouchard 65 oil barge: petroleum hydrocarbons persist in Winsor Cove salt marsh sediments. Mar. Pollut. Bull. 54, 2214–2225.
Pennings, S.C., McCall, B.D., Hooper-Bui, L.M., 2014. Effects of oil spills on terrestrial arthropods in coastal wetlands. Bioscience (in press).
Pritchard, P.H., Costa, C.F., 1991. EPA's Alaskan oil spill bioremediation project. Environ. Sci. Technol. 25, 372–379.
Qimin, M.A., Xia, L., Zhigang, Y., 2007. Changes in the chemical components of light crude oil during simulated short term weathering. J. Ocean Univ. China 6, 231–236.
Reddy, C.M., Eglinton, T.I., Hounshell, A., White, H.K., Xu, L., Gaines, R.B., Frysinger, G.S., 2002. The West Falmouth oil spill: the persistence of petroleum hydrocarbons in marsh sediments. Environ. Sci. Technol. 36, 4754–4760.
Reddy, C.M., Arey, J.S., Seewald, S., Sylva, S.P., Lemkau, K.L., Nelson, R.K., Carmichael, C.A., McIntyre, C.P., Fenwick, J., Ventura, G.T., 2012. Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. Proc. Natl. Acad. Sci. (USA) 109, 20229–20234.
Sverdrup, L.E., Nielsen, R., Krough, P.H., 2002. Soil ecotoxicity of polycyclic aromatic hydrocarbons in relation to soil sorption, lipophilicity, and water solubility. Environ. Sci. Technol. 36, 2429–2435.
Tattersfield, F., 1928. The decomposition of naphthalene in the soil, and the effect upon its insecticidal action. Ann. Biol. 15, 57–80.
Teal, J.M., Farrington, J.W., Burns, K.A., Stegeman, J.J., Tripp, B.W., Woodin, B., Phinney, C., 1992. The West Falmouth oil spill after 20 years: fate of fuel oil compounds and effects on animals. Mar. Pollut. Bull. 24, 607–614.
Turner, R.E., Overton, E.B., Ashton, B.M., Miles, M.S., McClenachan, G., Hooper-Bui, L., Summer Engel, A., Swenson, E.M., Lee, J.M., Milan, C.S., Gao H., 2014. Distribution and recovery trajectory of Macondo (Mississippi Canyon 252) oil in Louisiana salt marshes. Mar. Pollut. Bull. (in preparation).
Turner, R.E., Swenson, E.M., Milan, C.S., 2000. Organic and inorganic contributions to vertical accretion in salt marsh sediments, pp. 583–595. In: Weinstein, M., Kreeger, D.A. (Eds.), Concepts and Controversies in Tidal Marsh Ecology. Kluwer Academic Publishing, Dordrecht, Netherlands, p. 864.
US EPA (United States Environmental Protection Agency), 2007. Test methods for evaluating solid wastes, physical/chemical methods, SW-846. USEPA, Office of Solid Waste and Emergency Response. Washington, D.C. <http://www.epa.gov/epawaste/hazard/testmethods/index.htm>.
Venosa, A.D., Suidan, M.T., Wrenn, B.A., Strohmeier, K.L., Haines, J.R., Eberhart, B.L., King, D., Holder, E., 1996. Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. Environ. Sci. Technol. 30, 1764–1775.
Ward, D.M., Atlas, R.M., Boehm, P.D., Calder, J.A., 1980. Microbial biodegradation and chemical evolution of oil from the Amoco spill. Ambio 9, 277–283.
Whitehead, A., Dubansky, B., Bodinier, C., Garcia, T.I., Miles, S., Pilley, C., Raghuathan, V., Roach, J.L., Walker, N., Walter, R.B., Rice, C.D., Galvez, F., 2012. Genomic and physiological footprint of the Deepwater Horizon oil spill on resident marsh fishes. Proc. Natl. Acad. Sci. (USA) 109, 20298–20302.