

Incorporation of Deepwater Horizon oil in a terrestrial bird

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LETTER

Incorporation of Deepwater Horizon oil in a terrestrial bird

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A Bonisoli-Alquati¹, P C Stouffer¹, R E Turner², S Woltmann³ and S S Taylor¹¹ School of Renewable Natural Resources, Louisiana State University and LSU AgCenter, USA² Department of Oceanography and Coastal Sciences, Louisiana State University, USA³ Department of Biology and Center of Excellence for Field Biology, Austin Peay State University, USAE-mail: andreabonisoli@gmail.com**Keywords:** deepwater horizon, Macondo oil, oil pollution, oil spill, radiocarbon, environmental forensics**Abstract**

Carbon isotopic evidence revealed Deepwater Horizon (DWH) oil entering coastal planktonic and lower terrestrial food webs. The integration of spilled oil into higher terrestrial trophic levels, however, remains uncertain. We measured radiocarbon (^{14}C) and stable carbon (^{13}C) in seaside sparrow (*Ammodramus maritimus*) feathers and crop contents. Lower ^{14}C and ^{13}C values in feathers and crop contents of birds from contaminated areas indicated incorporation of carbon from oil. Our results, although based on a small sample of birds, thus reveal a food-web link between oil exposure and a terrestrial ecosystem. They also suggest that the reduction in reproductive success previously documented in the same population might be due to the (direct) toxic effect of oil exposure, rather than to (indirect) ecological effects. We recommend future studies test our results by using larger samples of birds from a wider area in order to assess the extent and implications of DWH oil incorporation into the terrestrial food web.

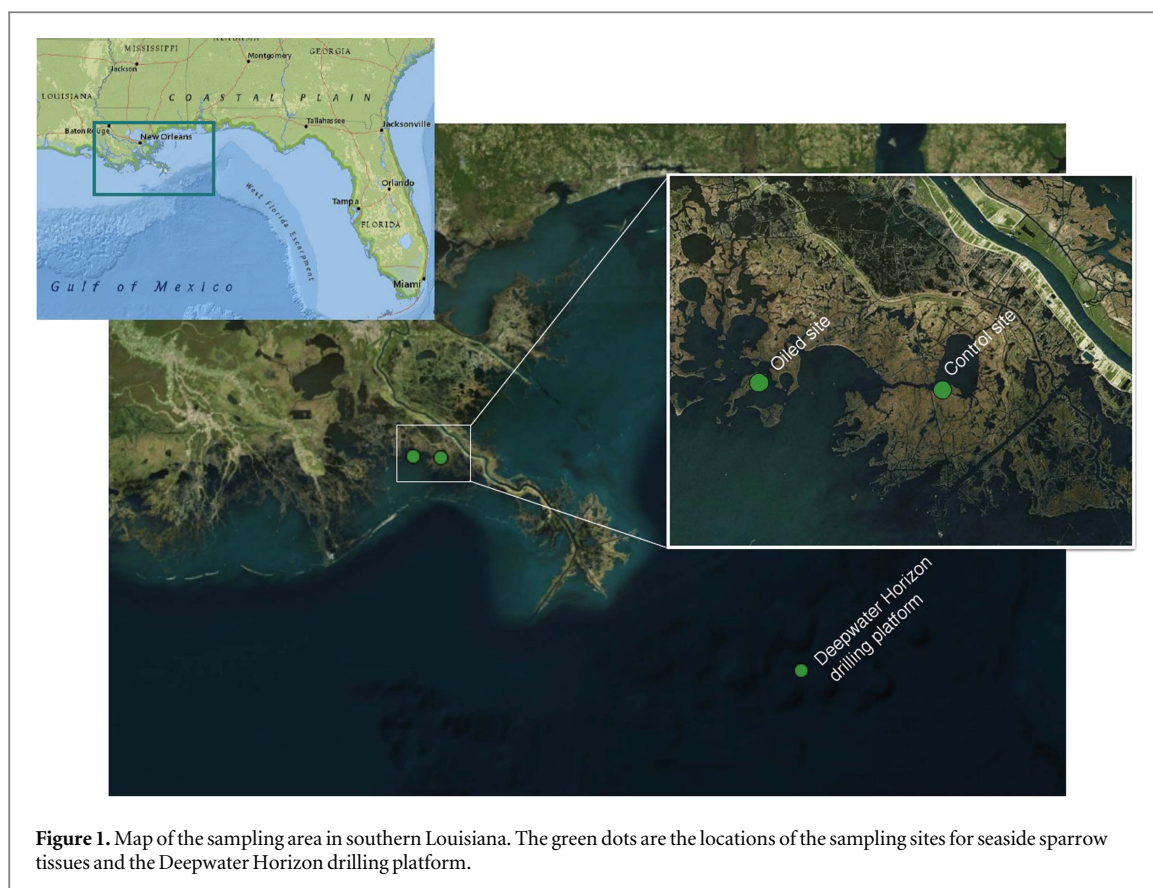
Introduction

The 2010 Deepwater Horizon (DWH) oil spill released up to 700 000 m³ of oil (Crone and Tolstoy 2010) and 500 000 t of gaseous hydrocarbons (Joye *et al* 2011) into the northern Gulf of Mexico over 87 days. It is estimated that 10%–29% of the spilled oil was chemically dispersed, 20%–25% evaporated or dissolved, 23%–27% was recovered at the well head, burned or skimmed at the surface, while 12%–13% naturally dispersed (Lehr *et al* 2010). The remaining oil was at least partly deposited on the seafloor (Chanton *et al* 2015), or reached coastal marshes and beaches of the northern Gulf of Mexico (Joye 2015).

Several studies have since attempted to trace the ecological fate of spilled DWH oil. Fossil carbon, such as oil, completely lacks radiocarbon (^{14}C) because of its geologically short 5730-yr half-life. Oil carbon is also depleted in stable carbon (^{13}C) compared to surface production in the open ocean. The incorporation of oil carbon into an ecosystem, therefore, may be tracked by measuring the depletion in ^{14}C and/or ^{13}C that results from the admixture of oil carbon and carbon derived from surface water primary production (White *et al* 2005).

A metagenomic analysis of microbial communities demonstrated that they had responded to the release of oil (Redmond and Valentine 2012), thus making it potentially available to higher trophic levels. A stable carbon ($\delta^{13}\text{C}$) analysis has demonstrated that subsurface DWH oil carbon entered zooplanktonic communities soon after the spill (Graham *et al* 2010). Other radiocarbon analyses confirmed this finding in the planktonic food web of the open waters of the Gulf of Mexico (Chanton *et al* 2012). However, the extent to which DWH oil was incorporated into the coastal food web is less clear. Depleted radiocarbon values in the tissues of coastal invertebrates and fishes indicated that DWH oil was assimilated by coastal organisms (Wilson *et al* 2016). Yet only a minimal trace of DWH oil seemed to have reached estuarine filter-feeding barnacles (*Balanus* sp.) and marsh mussels (*Geukensia demissa*) (Fry and Anderson 2014). Moreover, oysters from coastal waters of Mississippi contaminated by the DWH oil did not show any evidence of oil incorporation into their shells or soft tissues (Carmichael *et al* 2012).

We know of no reports of DWH oil in tissues of entirely terrestrial animals; such a link would demonstrate contamination into higher trophic levels beyond



direct contact with oil. We screened for oil carbon assimilation at higher trophic levels by investigating hydrocarbon-related changes in carbon isotopic composition in a terrestrial bird, the seaside sparrow (*Ammodramus maritimus*). We measured radiocarbon and stable carbon content in feathers and crop contents collected from birds in 2011 from sites that were oiled or left uncontaminated by the DWH oil spill. We also analyzed coastal sediments collected in 2011 in nearby locations that were reached by Macondo 252 (MC252) oil, and conducted oil source-fingerprinting.

Methods

Seaside sparrow sample collection

The seaside sparrow is a year-round resident of Louisiana marshes requiring highly specific habitat for foraging and nesting (Post *et al* 1983). The seaside sparrow is socially monogamous, and both parents feed their chicks during the nine days from hatching to fledging, as well as for a few days after fledging (Post *et al* 1983).

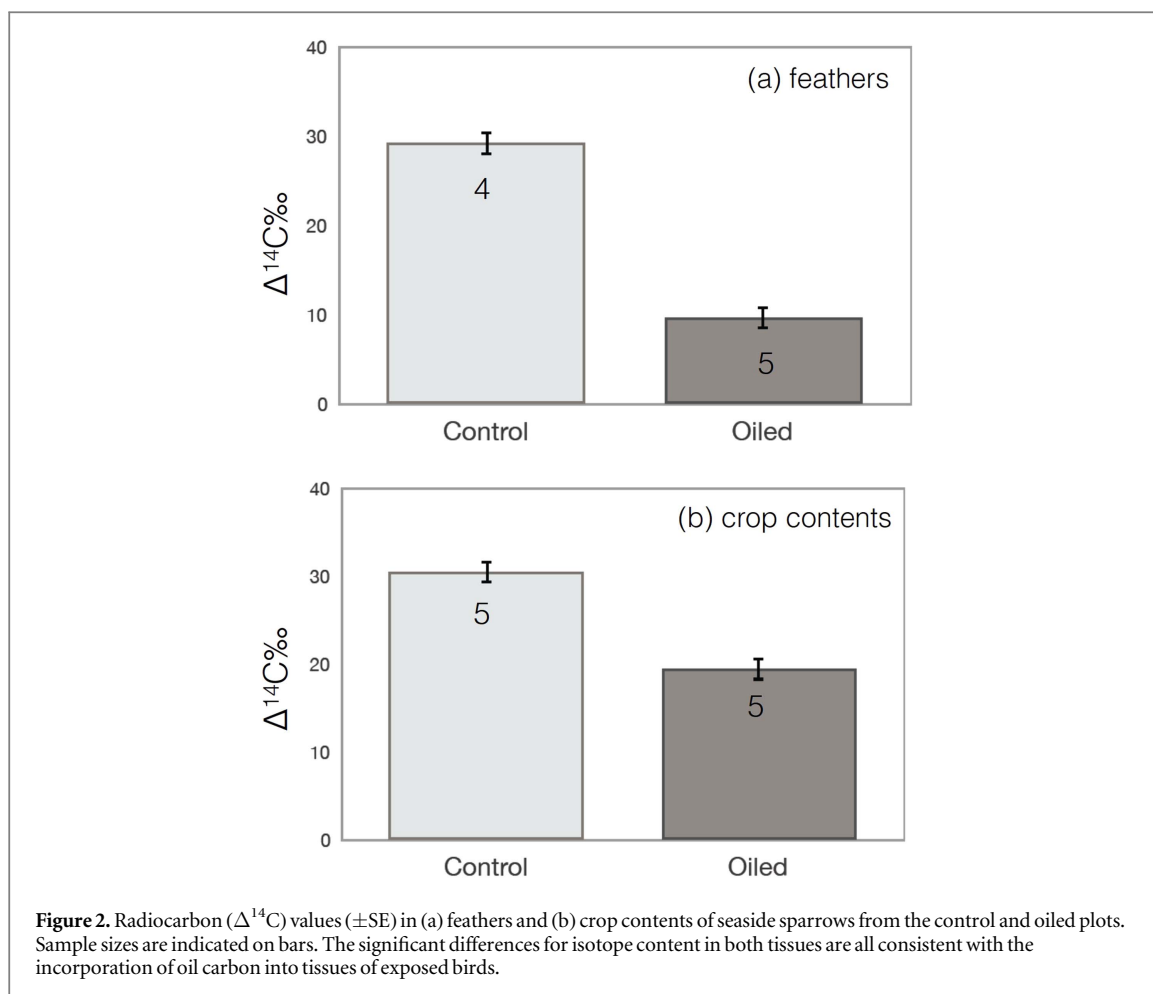
Adults and juveniles both feed on a variety of prey, including terrestrial as well as marine invertebrates (Post and Greenlaw 2006), thus exposing this species to a variety of contaminants through a number of potential routes. Consistent with this, the seaside sparrow was found to be a sensitive indicator species for

mercury contamination in salt marshes (Warner *et al* 2010, Winder 2012).

We collected ten seaside sparrows (eight juveniles, two adults) in August 2011 with a shotgun loaded with 22 dust (collection permits: USFWS MB679782; LDWF LNHP11-068) on two sites (four juveniles and one adult bird each; figure 1) that we classified as oiled or uncontaminated (control) based on Shoreline Cleanup and Assessment Technique (SCAT) surveys maps (<http://gomex.erma.noaa.gov/erma.html#/x=-89.37870&y=29.14486&z=7&layers=16+6770+15879+19872+19897>) (Santner *et al* 2011). In order to control for the potential confounding effect of sex, we molecularly sexed all birds according to established protocols. All birds were male, with the exception of two females from the control site. Crop contents were flash-frozen using liquid nitrogen in the field following terminal collection. One or two primary or secondary feathers were collected from each bird.

Sample preparation and carbon isotopic analyses

Feathers and crop contents were analyzed for their carbon isotopic composition at the Rafter Radiocarbon Laboratory (Lower Hutt, NZ), where their radiocarbon content was measured using accelerator mass spectrometry. The feathers and crop contents were combusted using a combination of either elemental analysis combustion or sealed tube combustion methods. Feather samples were cleaned with washes of hexane, isopropanol, and acetone, while no



surface cleaning was performed on crop contents. We dried crop contents in a convection oven at 60 °C, and then pulverized them using an automated shaker and stainless steel capsules and ball bearings.

Carbon dioxide (CO_2) gas was produced from the pretreated residue of samples and purified for graphitization. Samples were combusted at 900 °C for 4 h in evacuated, sealed quartz tubes with cupric oxide and silver wire (Turnbull *et al* 2014). The cupric oxide provides oxygen for the combustion and the silver isolates sulfur and halogens in a solid form. CO_2 was cryogenically purified after combustion by passing it through traps of ethanol/dry ice to remove water. The purified CO_2 was then collected in a glass vessel for transport to the graphitization and mass spectrometry laboratories.

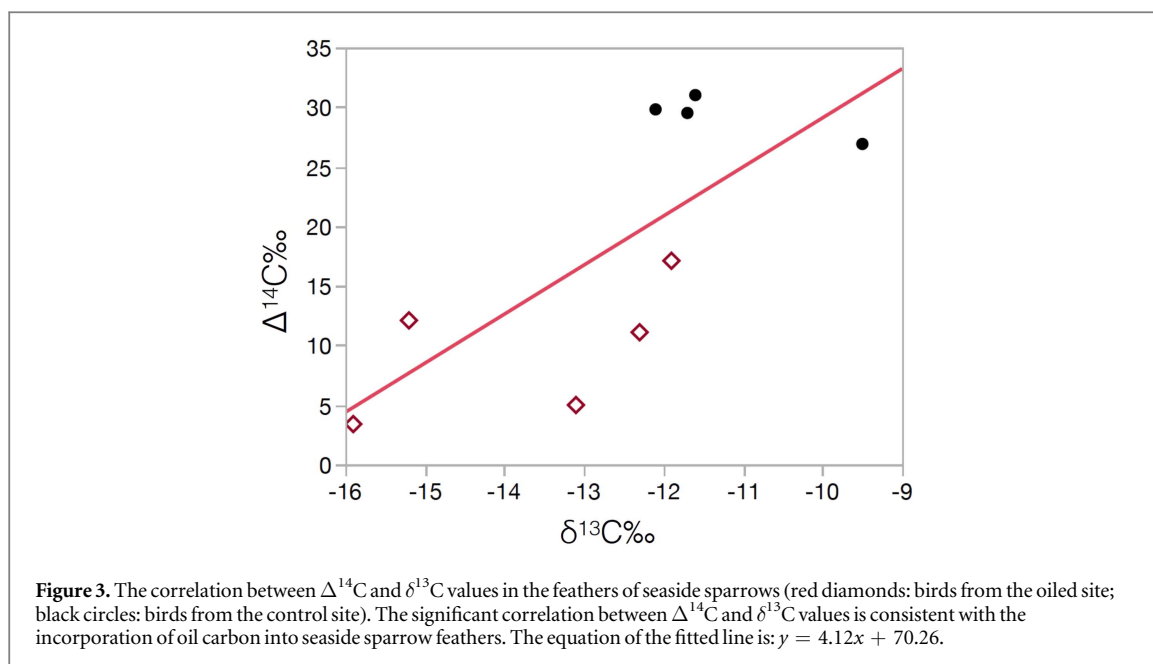
^{14}C measurements are expressed using the notation $\Delta^{14}\text{C}$, which is the per mille (‰) deviation from an oxalic acid international standard for radiocarbon dating (Stuiver and Polach 1977).

Sediment sample collection and oil source-fingerprinting

In order to confirm the information from the SCAT maps, we collected sediments from each site, and determined stable carbon and radiocarbon values for these samples. Sediment 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). Sample analysis is described in detail in Turner *et al* (2014), which discusses changes in the total alkanes and total polycyclic aromatic hydrocarbons (PAHs) in time and space for the study area. In brief, we 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 C23 and C31) (Turner *et al* 2014). A daily calibration standard and blank were analyzed with each sample batch to verify proper instrument performance. 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).

We conducted oil source-fingerprinting using diagnostic ratio analysis of petroleum biomarkers, according to an established gas chromatographic-



mass spectrometer (GC/MS) methodology (Daling *et al* 2002, Hansen *et al* 2006), which had been previously validated for Macondo 252 (MC252) oil (Meyer *et al* 2014, Ramsey *et al* 2014). Petroleum biomarkers are compounds that are ubiquitous across different crude oils, resistant to degradation, and specific to the oil's biogenic precursors (Wang *et al* 2006). We used pattern recognition and retention times of specific hopane, sterane, and triaromatic steroid cyclic biomarkers to determine eleven quantitative diagnostic indices (ratios) between target samples and a MC252 reference standard. We classified oil detected in a sediment sample as a positive match with MC252 oil only if all 11 diagnostic ratios consistently indicated a match.

Statistical tests for the difference between birds in the control and oiled sites were run as one-way ANOVA. Due to the small sample size, and uneven age and sex composition in the two experimental groups, we could not test the two-way interaction effects between treatment, age and sex. The associations between radiocarbon and stable carbon values were tested using simple regression analyses.

Results

The average radiocarbon value ($\Delta^{14}\text{C}$) was 18.4‰ (3.7 SE) in feathers, and 25.1‰ (2.0 SE) in crop contents. The radiocarbon content was lower in the feathers as well as in the crop contents of birds from oiled compared to control plots (figures 2(a) and (b)), which is consistent with the incorporation of DWH oil into the tissues of exposed birds.

The average stable carbon ($\delta^{13}\text{C}$) value was -12.6 ‰ (0.7 SE) in feathers and -15.0 ‰ (0.7) in crop contents. Sparrow feathers from the oiled site had lower $\delta^{13}\text{C}$ values than those from unexposed

birds ($\delta^{13}\text{C}$ control: -11.2 ‰ (0.7 SE); oiled: -13.7 ‰ (0.7 SE); $F_{1,7} = 5.6$; $p = 0.0499$, $N = 9$). The $\delta^{13}\text{C}$ values for crop contents did not differ between birds from control and oiled sites ($\delta^{13}\text{C}$ control: -13.8 (0.8 SE); oiled: -16.1 (1.1 SE); $F_{1,8} = 2.92$; $p = 0.126$, $N = 10$).

When we restricted the analyses to the juvenile birds, the radiocarbon values were significantly lower in birds from the oiled site, in the feathers (control site: 28.7‰ (2.9 SE), oiled site: 9.4‰ (2.5 SE); $F_{1,6} = 25.22$, $P = 0.004$, $N = 7$) as well as the crop contents (control site: 30.18‰ (1.45 SE), oiled site: 18.73‰ (1.45 SE), $F_{1,7} = 31.19$, $P = 0.0014$, $N = 8$). $\delta^{13}\text{C}$ values, however, did not differ between the two groups for either feathers (control site: -11.1 ‰ (1.0 SE), oiled site: -14.0 ‰ (0.8 SE); $F_{1,6} = 5.17$, $P = 0.072$, $N = 7$) or crop contents (control site: -14.1 ‰ (0.7 SE), oiled site: -15.1 ‰ (0.7 SE); $F_{1,7} = 0.94$, $P = 0.371$, $N = 8$). Results were qualitatively unchanged when we excluded the two females in order to check for the potential confounding effect of sex on the difference between the oiled and control sites (details not shown).

The $\Delta^{14}\text{C}$ and $\delta^{13}\text{C}$ values were positively correlated in feathers (slope (SE) = 4.12 (1.49); $R^2 = 0.45$, $p = 0.028$, $N = 9$; figure 3), but not in crop content samples (slope (SE) = 1.19 (0.89); $R^2 = 0.08$, $p = 0.216$, $N = 10$). Irrespective of the location, $\Delta^{14}\text{C}$ values for feathers and crop contents were significantly and positively correlated to each other (slope (SE) = 1.74 (0.27); $R^2 = 0.83$, $p = 0.0004$, $N = 9$), while this was not the case for stable carbon values (slope (SE) = 0.35 (0.19); $R^2 = 0.23$, $p = 0.11$, $N = 9$). Finally, there was a strong positive relationship between $\delta^{13}\text{C}$ values in feathers and $\Delta^{14}\text{C}$ values in the crop contents (slope (SE) = 0.27 (0.07); $R^2 = 0.61$, $p = 0.0083$, $N = 9$), which is somewhat

puzzling given the different timescales that are indicated by the two tissues.

The sediments from control compared to oiled locations did not differ in either stable carbon isotopes ($\delta^{13}\text{C}$ control: -18.4‰ (2.2 SE); oiled: -14.5‰ (0.4 SE); $F_{1,8} = .303$; $p = 0.120$, $N = 10$), or radiocarbon content ($\Delta^{14}\text{C}$ control: 19.4‰ (29.5 SE); oiled: 120.0‰ (65.3 SE); $F_{1,8} = 1.97$; $p = 0.198$, $N = 10$). The stable carbon values of sediments were positively associated with the radiocarbon values, although the relationship was marginally non significant (slope (ES) = 16.99 (8.91); $R^2 = 0.31$, $p = 0.093$).

In three of five sediment samples from the oiled site the diagnostic ratios of the eleven biomarkers confirmed the presence of MC252 oil. In the other two samples, our analyses yielded inconclusive or negative evidence, with only ten or eight out of eleven biomarkers matching MC252 oil, respectively. In none of the five sediment samples from the control site did the diagnostic ratios indicate the presence of MC252 oil.

Discussion

Our analyses of carbon isotopic composition of seaside sparrow tissue indicated incorporation of oil carbon into the tissues of birds collected from an area reached by DWH oil. To our knowledge, this is the first demonstration that we know of where DWH oil was incorporated into a terrestrial vertebrate species. Importantly, data from the same population of seaside sparrows examined here indicated that reproductive success was reduced in birds from oiled plots in the early years after the spill (Bergeon Burns *et al* 2014), although reproductive success seemed to recover in subsequent years. In principle, such reduction of population fitness could be due to the indirect effects of oil contamination on the salt marsh ecosystem. Such indirect effects include a reduction in habitat quality as well as in the abundance of insects and other invertebrates that are part of the seaside sparrow diet. Consistent with this interpretation, several studies have shown population declines in insects, spiders and other invertebrates in sites oiled as a result of the DWH accident (McCall and Pennings 2012, Zengel *et al* 2015, Husseneder *et al* 2016, Zengel *et al* 2016). DWH oil also impacted vegetation cover (Zengel *et al* 2015), including plant species (particularly *Spartina alterniflora*, *Distichlis spicata* and *Juncus roemerianus*) used by seaside sparrows as nesting habitat, thus potentially affecting the outcome of reproductive attempts. Because we could trace the direct incorporation of DWH oil into the tissues and prey of seaside sparrows, the present results suggest that direct toxicological effects, not only habitat degradation or trophic interactions, might be responsible for the demonstrated reduction in seaside sparrow reproductive success.

Furthermore, the oil incorporation and direct toxic effects are consistent with our finding of increased expression of cytochrome P-4501A (CYP1A), a marker of exposure to PAHs. CYP1A expression in 2011 was higher in birds from sites reached by DWH oil versus unoiled sites (Bergeon-Burns, Stouffer, Taylor, Woltmann, in review), echoing findings from marine organisms (Whitehead *et al* 2012).

Importantly, the direct (i.e. toxicological) and indirect (i.e. ecological) pathways of effect of DWH oil on seaside sparrow populations are not mutually exclusive. Rather, they are expected to compound in additive or synergistic ways to ultimately determine fitness reductions in exposed organisms (Whitehead 2013). Future studies might characterize how oil-derived stress propagated through the coastal food web, and impacted higher trophic levels. In seaside sparrows, this will imply analyzing reproductive success while also controlling for food abundance as well as vegetation composition and cover in both oiled and control sites. Since our study only analyzed ten individual birds, we could not examine multiple locations from a wider region or explicitly analyze variation in exposure due to age and sex, a task that is left to future studies with larger sample sizes. Following seaside sparrows of known sex through reproduction will also clarify whether offspring of exposed parents are at risk due to maternal transfer of contaminants through the egg.

Interestingly, the depletion of radiocarbon in birds from oiled sites was greater for feathers than for crop contents (figure 2). As for many songbirds, feather development in juvenile seaside sparrows continues for a few days after fledging (9–10 days of age). The feathers, therefore, incorporate carbon from food fed by seaside sparrow parents, as well as from items that the fledglings obtained independently. The information provided by feathers, therefore, encompasses more life stages, locations and time compared to information from an analysis of crop contents. In spite of this, our results indicate that the carbon isotopic composition of feathers provides a sensitive, integrated measure of oil carbon assimilation by birds over longer timescales than from crop contents alone, with the additional benefit that feathers can be collected without the need to euthanize the birds.

Oil source fingerprinting in sediments from the same site where we sampled the exposed birds indicated the presence of DWH oil. Alternative explanations for the depletion of radiocarbon in seaside sparrow tissues are therefore unlikely. Wilson *et al* (2016) suggested that the decline in ^{14}C values that they detected in the Eastern Gulf of Mexico in a variety of fish and invertebrate tissue and shell samples could be due to the input of radiocarbon-depleted dissolved inorganic carbon from the karst system of Western Florida. Given the different geology of our study sites,

this explanation does not apply to our findings. Moreover, the demonstrated positive relationship between stable carbon values and radiocarbon values in feathers from seaside sparrows indicated that radiocarbon depletion was due to oil carbon contribution, rather than to freshwater input (Chanton *et al* 2012).

We detected a small, but statistically significant depletion in stable carbon values in feathers ($\sim 2\%$) of exposed seaside sparrows compared to unexposed birds. This difference is one order of magnitude smaller than the one detected using radiocarbon ($\sim 20\%$). The difference in stable carbon values in crop contents, instead, was not statistically significant. Thus, our results confirm that the higher sensitivity of radiocarbon analyses compared to stable carbon analyses might be needed to detect oil incorporation into biological matrices, particularly in organisms that come in contact with oil through their diet. Given the absence of radiocarbon from oil, the organic production in the ocean differs from DWH oil by 1000‰ for radiocarbon (Fry and Anderson 2014), as opposed to only 5–7‰ for stable carbon (Graham *et al* 2010).

Conclusions

We documented statistically significant differences in radiocarbon values between the tissues of exposed and control birds following the DWH oil spill. These results are consistent with the incorporation of DWH oil into the tissues of the exposed birds, and provide the first evidence that the oil made it into the terrestrial food web. The documented differences are admittedly small, corresponding to $\sim 2\%$ oil carbon incorporation in feathers and $\sim 1\%$ in crop contents. These small contributions are in line with other findings in organisms that are taxonomically and ecologically distant from the seaside sparrow (Chanton *et al* 2012, Fry and Anderson 2014). Our data indicating a decline in reproductive success of exposed birds in 2012 and 2013 (Bergeon Burns *et al* 2014) suggest that the toxicological significance of such small incorporation should not be underestimated.

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the Gulf of Mexico Research Initiative Information and Data Cooperative (GRIIDC) at <https://data.gulfresearchinitiative.org> (doi: 10.7266/N7D21VN2).

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