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Assimilation of Oil-Derived Elements by Oysters Due to the Deepwater Horizon Oil Spill

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Supporting Information

ABSTRACT: During and after the Deepwater Horizon Oil Spill (DWHOS), oysters (*Crassostrea virginica*) were exposed to oil and susceptible to incidental consumption of surface and subsurface oil materials. We determined the contribution of oil materials from the DWHOS to diet of oysters by comparing carbon (C) and nitrogen (N) stable isotope ratios in oyster shell to ratios in suspended particulate matter (SPM) and in fresh and weathered oil. Average δ^{13}C and δ^{15}N values in oyster shell (−21 ± 1‰ and 9−11‰, respectively) were consistent with consumption of naturally available SPM as opposed to values in oil (−27 ± 0.2‰, 1.6 ± 0.4‰). Stable isotope ratios in oyster adductor muscle were similar to shell for δ^{15}N but not δ^{13}C, suggesting either a recent shift in diet composition or differential assimilation of C between tissue types. We found no evidence of assimilation of oil-derived C and N and, therefore, no evidence of an oyster-based conduit to higher trophic levels. Trace elements in shell were inconclusive to corroborate oil exposure. These findings are not an indication that oysters were not exposed to oil; rather they imply oysters either did not consume oil-derived materials or consumed too little to be detectable compared to natural diet.

INTRODUCTION

Between April 20 and July 15, 2010, approximately 500 000 tons of crude oil spilled into the northern Gulf of Mexico.[1] Often referred to as the worst environmental disaster in America's history, the Deepwater Horizon oil spill (DWHOS) was expected to significantly alter the Gulf of Mexico marine ecosystem with potentially long-term effects to coastal and open waters.[1,2] In many cases, however, the extent and nature of effects have been difficult to quantify due to the physical setting, offshore application of dispersants, potentially rapid microbial degradation, and low detection rates for affected organisms.[1,3,4] Additionally, much of the oil material that flowed from the well was natural gas and very light hydrocarbons that dissolved in the water column without entering surface waters of nearshore environments.[5,6]

While there has been significant consideration to physical transport, microbial degradation, and direct toxicity of oil-derived products from the DWHOS,[1,7,8] potential effects on local food webs have been largely overlooked.[7] Biota in the northern Gulf of Mexico was potentially exposed to relatively fresh surface oil during the spill as well as to weathered oil that settled to the bottom and continues to intermittently appear on local beaches and subtidally (Figure 1). As a major organic carbon (C) and nitrogen (N) source, oil-derived substances from the DWHOS had potential to feed secondary production or shift food web structure to favor species able to utilize those resources. Assimilation of oil-derived elements into local food webs could also provide an alternate pathway of oil degradation that has not yet been defined.

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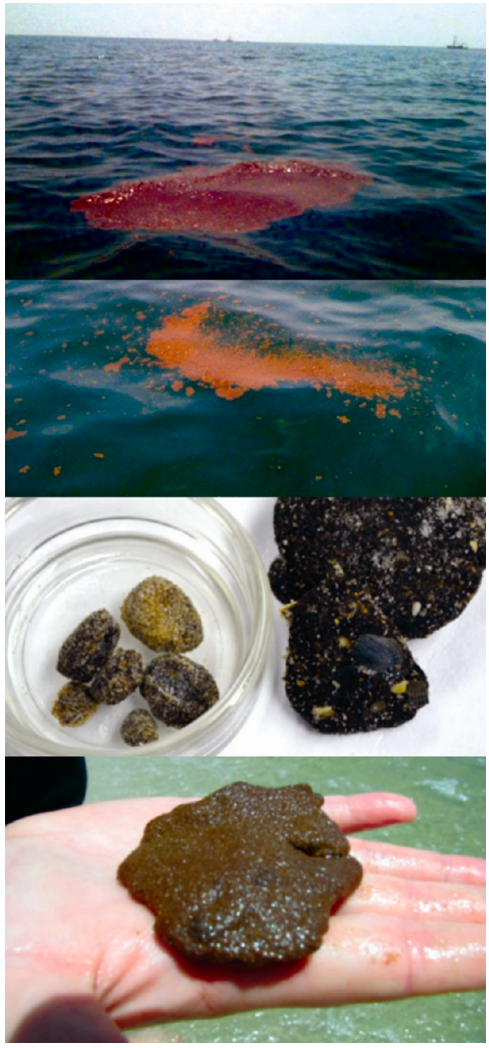

Figure 1. Weathered surface oil in the northern Gulf of Mexico approximately 20 km south of Dauphin Island, Alabama in early June 2010 (top panels) and examples of "balls" or "patties" of weathered oil collected from local beaches and near shore areas along the Florida–Alabama–Mississippi coast from June 2010 to July 2011 (bottom panels).

In the northern Gulf of Mexico, oyster reefs and areas historically productive for oysters were potentially exposed to oil for several weeks during the DWHOS. The eastern oyster, *Crassostrea virginica*, is economically and ecologically valuable and ubiquitous along the northern Gulf of Mexico coast, making it of high interest for local study related to the effects of the DWHOS.[7,9] Oysters are sessile suspension-feeders that live and feed in near-bottom waters and assimilate particles that reflect the surrounding environment. This lifestyle rendered oysters susceptible to incidental consumption of surface and subsurface oil during and after the DWHOS. Like other bivalves, as *C. virginica* grows, it assimilates organic and inorganic material into its shell as well as into soft tissue.[10,11] Organic material within the shell is embedded in a calcium carbonate-based matrix deposited in relatively discrete increments and less affected by subsequent metabolic processes than soft tissues.[12] Oysters also bioaccumulate trace metals into tissue and shell,[13] some of which may be useful to detect exposure to oil pollution.[14,15] Hence, oysters are potentially powerful recorders of environmental variation, particularly changes in food sources and ambient water quality. These attributes make oysters useful sentinels of oil entry into the local food web.

In this study, we applied stable isotope analysis to define the contribution of oil-derived C and N to the diet of a locally important primary consumer (*C. virginica*), during and after the DWHOS. We specifically compared C and N stable isotope ratios in oyster shell (deposited during discrete time intervals before, during, and after the DWHOS) to stable isotope ratios in suspended particulate matter (SPM) available as food to oysters and in fresh and weathered oil. Stable isotope ratios are commonly used to define diets and associated food web linkages because physiological processes result in relatively consistent fractionation of stable isotope ratios from food source to consumer ($\sim$ +2–4‰ for N and $\pm$1‰ for C[16,17]). The relatively conservative fractionation of C facilitates identification of food sources with discrete organic C stable isotope ratios at the base of food webs.[17] We hypothesized that oil-derived elements would be detectable in the new growth at the shell margin of oysters growing in local waters during and after the DWHOS, if oil-derived substances were consumed in nutritionally significant quantities. We also hypothesized that organic material in the oyster shell would be a more reliable indicator of oil assimilation than soft tissues because shell is deposited in discrete layers during the time of exposure, while soft tissue reflects a mixture of recent and previously consumed foods. To corroborate our findings and detect oil exposure even if oysters did not consume oil-derived materials, shells were analyzed for shifts in concentration of trace and minor elements that may be associated with exposure to oil materials.

EXPERIMENTAL SECTION

Shells were collected from oysters transplanted at different locations (along east–west and north–south trajectories on the coast) and time periods (before, during, and after) relative to the MC 252 Deepwater Horizon oil spill (Figure 2 and Table 1). This approach allowed us to compare temporally and spatially explicit stable isotope shifts recorded in oyster shell relative to potential oil exposure through time.

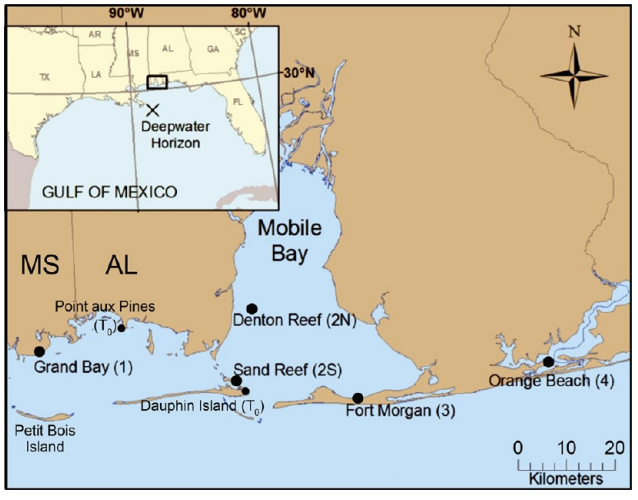

Figure 2. Study sites where oysters were deployed in Mobile Bay and surrounding waters along the Mississippi (MS)–Alabama (AL) coast. Site numbers correspond to data in Table 1. The inset shows the location of study sites relative to the Deepwater Horizon oil rig (×).

Table 1. Estimated Oil Exposure for Time Zero (T_0) and Transplanted Oysters in Mobile Bay (Denton Reef and Sand Reef) and along the MS−AL Coast (Grand Bay, Fort Morgan, Orange Beach)[a]

		T_0		treatment	
site	site	none[b]	unlikely[c]	possible exposure[d]	postspill exposure[e]
Grand Bay	1		30 Apr 2010	1 Jul 2010	6 Oct 2010
Denton Reef	2N	30 June 2008	14 May 2010	21 Jul 2010	3 Aug 2010
Sand Reef	2S	30 June 2008	14 May 2010	20 Jul 2010	4 Aug 2010
Fort Morgan	3		30 Apr 2010	30 Jun 2010	7 Oct 2010
Orange Beach	4		30 Apr 2010	30 Jun 2010	7 Oct 2010

[a]Site numbers correspond to locations shown in Figure 2 and Supporting Information Figure 1. [b]Prespill; hatchery stock held on Dauphin Island at a site central to coastal and Mobile Bay transplant sites prior to the DWHOS. [c]During spill; hatchery stock held at Point aux Pines prior to transplanting, during active spilling of oil, but prior to documented presence of oil along the coast. [d]During spill; oil reported along the Mississippi−Alabama coastline, surface and subsurface oil exposure possible. [e]Postspill; Deepwater Horizon well was shut down, surface oil was absent from the Mississippi−Alabama coast, subsurface oil exposure possible.

Oyster Transplants. Hatchery-reared oysters from the Auburn University Shellfish Laboratory on Dauphin Island, Alabama were transplanted at two sites in Mobile Bay (Denton Reef = 2N, Sand Reef = 2S) and three sites along the Mississippi−Alabama coastline (Grand Bay = 1, Fort Morgan = 3, Orange Beach = 4) (Figure 2, Supporting Information SI-Table S1). In Mobile Bay, subadult oysters (42.1 ± 0.8 mm shell height, n = 50) were transplanted in plastic-coated wire mesh aquaculture cages measuring 33 × 33 × 10 cm deep and suspended 1.0 m above the sediment surface (n = 3 cages per depth per site). Oysters were planted in late May 2010 and collected in July (during spill) and August (postspill) 2010 (Table 1). The outer margin of each oyster shell was delineated with permanent marker prior to transplanting at these sites.[18,19] At coastal sites, subadult oysters (38.1 ± 1.0 mm shell height, n = 15) were transplanted in mesh bags similar to cages at Mobile Bay sites, but measuring 50 × 50 × 10 cm deep with 3.8 cm mesh (n = 4 bags per site). Cages loaded with bags were tethered to helix anchors and floated in 1.0 m of water. Oysters were deployed in May 2010 and collected from coastal sites in early July (during spill). New oysters were deployed in August and collected in early October (postspill). As a prespill control, we analyzed hatchery stock (Table 1, T_0) from late May 2008 (held at the Dauphin Island Sea Lab, a location central to both coastal and Mobile Bay sites) and hatchery stock held at Sandy Bay, Point aux Pines, Alabama (Figure 2) collected in early May 2010 for coastal sites and mid-May 2010 for Mobile Bay sites.

For analysis, T_0 and transplanted oysters were divided into categories based on likelihood of exposure to oil (Table 1). Categories (none, unlikely, possible exposure, postspill exposure) were defined based on the location of surface oil reported by NOAA[20] relative to the time period when oysters were transplanted at the field sites (SI-Figure S1).

Shell and Soft Tissue Sample Preparation. After collection, oyster shells were separated from soft tissues, thoroughly washed with ultrapure water, and dried under clean conditions in a fume hood at room temperature. To determine how stable isotope ratios in newly deposited shell compared to values in soft tissues (typically used for stable isotope analyses in trophic studies), adductor muscles were separated from whole tissues, cleaned with ultrapure water, and dried at 60 °C for analysis and comparison to shell.

The outer margin of new growth (deposited during field exposure) on each oyster shell was ground to a fine powder using a Dremel 300 Series hand-held rotary tool with a 1.3-mm ruby arbor band and collected in a watch glass.[11] The marginal cutting boundary was confirmed using the most conservative line based on comparison of direct growth measurements (with vernier calipers to the nearest 0.01 mm shell height), the macroscopic line of growth, and (in the case of Mobile Bay samples) a permanent marker line as independent indicators of the new growth boundary. To capture representative stable isotope values in shell material but maintain a reasonable sample number, powdered shell samples were aggregated from randomly selected oysters to produce two replicate aggregates of three or four oysters each from each site at each sampling period. We subsampled 250.0 ± 1.5 mg of each aggregate to prepare for stable isotope analysis.

To isolate the organic portion of the powdered shell material, we removed the inorganic fraction by acidifying each subsample using 0.5−1.0% $PtCl_2$ in 1 N HCl.[10,11] A volume of 2−4 mL of acid solution was added daily to the samples with gentle stirring until samples no longer reacted with the acid (bubbling ceased). Acidification typically required 2−3 days. After acidification, samples were filtered through preashed 0.7-μm Millipore glass-fiber filters. Filters were washed minimally with ∼5 mL ultrapure water, and dried to a constant weight at 60 °C.

Potential Food Sources. Because we made opportunistic use of oyster transplant experiments designed and initiated prior to the DWHOS, we did not have corresponding samples of suspended particulates matter (SPM) for every coastal site and sampling period. To determine stable isotope ratios in natural foods locally available to oysters, therefore, we utilized data from previous and ongoing studies at nearby sites during similar time periods, except in the cases of Denton and Sand Reef where site-specific data were collected every 2 weeks during the study period. In all cases, water was collected using a horizontal water sampler at locations of equivalent depth and salinity to study sites or (in the case of Mobile Bay sites) immediately adjacent to transplant cages (n = 31 Denton Reef; n = 33 Sand Reef). Water was prefiltered through a 200-μm mesh, vacuum filtered onto preashed 0.7-μm glass-fiber filters, and dried to a constant weight at 60 °C. For Grand Bay, we analyzed SPM data collected June−September 2010 from nearby Bayou La Batre, Alabama (n = 14). For Fort Morgan, we used SPM data collected in January and June 2010 from an adjacent site a few kilometers to the west on Fort Morgan (n = 14), and for Orange Beach, we used SPM data collected in June 2008 from a site within the intracoastal waterway, north of the transplant location (n = 13).

Weathered and Fresh Oil. To determine stable isotope composition of weathered oil, we sampled tar balls, mats, and semisolid oil forms (Figure 1) from sediments along the

shoreline from the Florida–Alabama border to Petit Bois Island in Mississippi (Figure 2) from June 2010 to July 2011. Weathered oil samples were analyzed from coastal areas near or at oyster transplant sites, including Petit Bois Island (near Grand Bay, n = 1), Dauphin Island (near Mobile Bay sites, n = 4), Gulf Shores (near Fort Morgan, n = 5), and Orange Beach (n = 2). Stable isotope ratios for fresher crude oil were determined in MC252 reference material obtained from BP Gulf Coast Restoration Organization (GCRO) in August 2011, including Massachusetts surrogate oil (MASS) and weathered oil from the surface (OFS), which are defined by BP GCRO as chemically and toxicologically similar to the Macondo Well in Mississippi Canyon Block 252. Subsamples from the interior of each weathered oil sample, MASS, and OFS (8–13 mg for C, 30–60 mg for N) were added dropwise to either 2.0 mg of CHROMOSORB WAW (ThermoFisher Scientific) for weathered oil samples or a preashed 0.7-μm glass-fiber filter for crude oil samples. Samples were incubated at 60 °C for up to 96 h to remove residual water prior to stable isotope analysis.

To confirm that weathered oil samples were derived from the DWHOS, we haphazardly selected samples from among the different locations of study and chemically fingerprinted 25% of the total number of weathered oil samples. Nonmatch samples were discarded along with any samples collected at the same time and location unless they were independently verified. Oil samples were sourced matched to MC252 oil by comparing the quantitative ratios of key markers (many of which are resistant to weathering) of petroleum hydrocarbons within the source oil to the same marker compounds in collected samples[21,22] (SI-Figure S2). Source oil (MC252 obtained from NOAA by E. Overton) and locally collected samples were dissolved in methylene chloride, and extracts were analyzed using capillary column gas chromatography–mass spectroscopy (GC-MS). Data were acquired in the selective ion mode. Ions and retention time windows were set to detect saturated hydrocarbons from C10 to C40 (ion 57), hopane and sterane tri to penta cyclic biomarkers (191, 217, 218, 231), and the following petroleum marker compounds and their alkyl homologues: naphthalene (ions 128, 142, 156, 170, 184), fluorene (166, 180, 194, 208), phenanthrene (178, 192, 206, 220, 234), dibenzothiophene (184, 198, 212, 226), benzo(a)anthracene, and chrysene (228, 242, 256, 270 284). Taking into consideration small daily instrumental variations and the fact that samples may contain different overall quantities of oily residues, we defined conclusive source identification when at least 90% of the biomarker compounds in the environmental sample matched the source oil (SI-Figure S2).

Stable Isotope Analysis. Samples were analyzed at the UC Davis Stable Isotope Facility by continuous flow–isotope ratio mass spectrometry (CF-IRMS; 20-20 mass spectrometer, PDZ Europa) after sample combustion to CO_2 and N_2 in an online elemental analyzer (PDZ Europa). Gases were separated on a Carbosieve G column (Supelco) before introduction to the CF-IRMS. As internal controls, blank filters and tins were analyzed along with an acetanilide standard (Fisher Scientific) of known isotope ratio and pseudoreplicates of randomly chosen samples, representing ~10% of the total sample number, to ensure variation of <0.2‰ due to sample handling and instrument reproducibility.

Trace Element Analyses. Oysters transplanted at coastal sites during possible and postspill exposure periods (Table 1) were analyzed for 26 trace and minor elements (Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Se, Sb, Ag, Sn, Si, Sr, Ti, V, Zn, K, Na), six of which have been highlighted for use in detection of hydrocarbon pollution (Cd, Co, Mo, Ni, Pd, V).[15,23,24] Shells were initially scrubbed in distilled deionized water with a soft brush to remove loosely attached biogenic and inorganic particles. Further cleaning was conducted, for each shell, with 5% weight to volume hydrogen peroxide (20 min), and rinsed with deionized distilled water (5 min). All samples were dried in a fume hood for 24 h. For each transplant period, three right valves of oyster shells were selected from each site and divided into three or four shell splits of equal length (~ 1.3 cm), depending on the length of the shell. The inner segments of shell toward the umbo region served as control samples because they corresponded to older growth in the hatchery or at T_0 sites before deployment, while the outer margin of shell represented new growth during the period of transplant. Old and new growth regions were identified using the independent measurements of shell growth described for stable isotope analyses. Samples were prepared by dissolving 35 mg of shell powder in 30 mL of a 10% HNO_3 solution and passing the resulting mixture through a 20-μm filter.[25] Major and minor elements were analyzed by inductively coupled plasma–optical emission spectrometry (ICP-OES) in the University of Alabama Department of Geological Sciences analytical geochemistry laboratory, with instrumental sensitivity of 10 ppb and ±8% error at 2σ. For all elements, a multielement inorganic calibration standard (High Purity Inc.) and quality control standards (CPI International) were used for analyses. Calibration was performed using concentrations 0.25, 0.5, 1.0, 5.0, 10.0, and 20.0 ppm (or mg L^{-1}) to within ±5%.

RESULTS

Stable Isotope Ratios in Oyster Shell. $\delta^{13}C$ values in newly deposited oyster shell averaged −20.8 ± 2.0‰, similar to $\delta^{13}C$ values typically found in marine phytoplankton (Figure 3A). $\delta^{13}C$ values in shell of most oysters grown in 2010 were lighter than those of prespill controls in 2008 (Figure 4), but there were no differences with level of potential exposure (ANOVA: F_2 = 0.001, P = 0.98), when estimated relative to the timing of transplants in the field in 2010 (Figure 4 and Table 1)

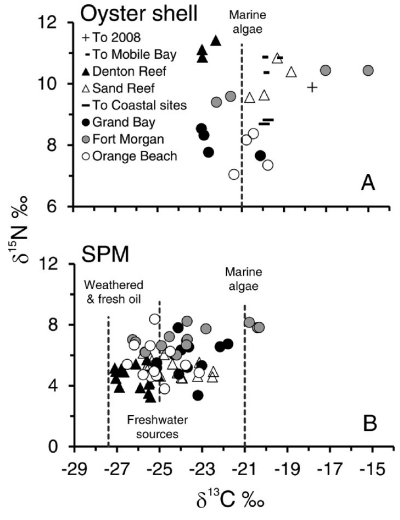

Figure 3. $\delta^{13}C$ and $\delta^{15}N$ ‰ in (A) oyster shell and (B) suspended particulate matter (SPM). Dashed lines represent average $\delta^{13}C$ values in marine algae, freshwater/terrestrial sources, and weathered and fresh oil (data from this study and others[17,28,42,43]).

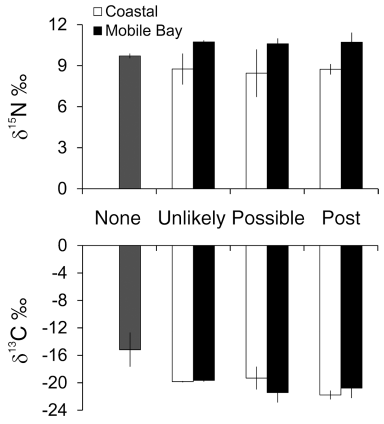

Figure 4. Mean (±se) δ^{13}C and δ^{15}N ‰ in shells of time zero and transplanted oysters at sites in Mobile Bay and along the Mississippi–Alabama coastline (Coastal), representing a range of potential exposure to oil materials from the DWHOS (defined in Table 1). The gray bar shows data from hatchery stock in 2008.

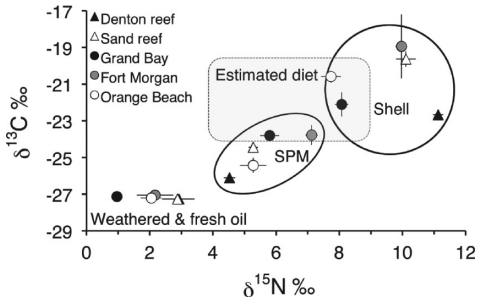

Figure 5. Comparison of mean (±se) δ^{13}C and δ^{15}N in oyster shells, SPM, and oil materials. The dashed box represents the expected range of δ ^{13}C and δ ^{15}N values in diet assimilated by oysters (assuming typical fractionation of ±1‰ for C, +2–4‰ for N). Circles define the range of values among sites for each sample type. Where no error bars are visible, error is smaller than the symbol.

or between oysters transplanted at Mobile Bay and coastal sites (ANOVA: F_1 = 1.60, P = 0.23). Oysters at the Fort Morgan site, however, showed an increase in δ^{13}C values compared to other sites during the post spill period (Figure 3A, values near −15‰ and −17‰, similar to 2008 prespill controls). N stable isotope values were heavier in shell of oysters from Mobile Bay sites compared to coastal sites (Figures 3A and 4), averaging 10.6 ± 0.2‰ and 9.4 ± 0.6‰, respectively (ANOVA: F_1 = 22.16, P < 0.001). δ^{15}N values in oyster shell during and after the DWHOS in 2010 were similar to those of prespill controls in 2008, and as with δ^{13}C there was no difference with level of potential exposure (ANOVA: F_2 = 0.30, P = 0.75) (Figure 4).

Stable Isotope Ratios in SPM and Oil Materials. Stable isotope values in suspended particulate matter available as food to oysters showed influence from both freshwater and marine sources (Figure 3B), but differed between Mobile Bay and coastal sites (ANOVA: δ^{13}C: F_1 = 6.41, P = 0.01; δ^{15}N: F_1 = 18.08, P < 0.001), showing lighter values in SPM from Mobile Bay sites (−25.3 ± 0.2‰ and 4.9 ± 0.2‰) compared to coastal sites (−24.3 ± 0.4‰ and 6.1 ± 0.3‰). Stable isotope ratios in weathered and fresher crude oil were lighter than values in SPM, averaging −27.4 ± 0.1‰ and 1.6 ± 0.4‰, respectively, for carbon and nitrogen (Figure 3B, SI-Table S2).

Comparison of Stable Isotope Ratios in Oysters to SPM and Oil Materials. Based on the stable isotope ratios we determined in oyster shell and typical fractionation for a single trophic step from food source to consumer, we estimated the isotopic values for assimilated diet of oysters at our transplant sites averaged approximately −21‰ for δ^{13}C and ranged roughly 4–9‰ for δ^{15}N (Figure 5). The actual mean difference between stable isotope ratios in oyster shell and SPM was 3.9 ± 2.1‰ for δ^{13}C and 3.8 ± 0.8‰ for δ^{15}N, generally consistent with a single trophic step. In contrast, the mean difference between stable isotope ratios in oyster shell and oil materials was nearly double compared to SPM, 6.4 ± 2.0 ‰ for δ^{13}C and 7.4 ± 1.0 ‰ for δ^{15}N (Figure 5).

Comparison between Oyster Shell and Soft Tissues. Stable isotope ratios in oyster shell and soft tissues differed for C but not N (SI-Figure S3). Stable isotope ratios in oyster adductor muscle averaged −23.4 ± 0.4‰ for δ^{13}C and 8.7 ± 0.6‰ for δ^{15}N, showing a difference of 1.3‰ and 3.4‰ for C and N, respectively, when compared to stable isotope ratios in SPM (values within the range of expected fractionation for a single trophic step). δ^{13}C values in shell and tissue were not correlated (Pearson Correlation: r = 0.50, P > 0.05), and tissue values were significantly lighter than values in shell (Mann–Whitney: U = 2.0, P = 0.01, two-tailed; median shell = −20.2‰, median tissue = −23.4 ‰; SI-Figure S3A). In contrast, δ^{15}N values in tissue and shell were highly correlated (Pearson Correlation: r = 0.97, P < 0.01), and values were not different in magnitude (Mann–Whitney: U = 15.0, P = 0.63, two-tailed; median shell = 9.7‰, median tissue = 8.4‰; SI-Figure S3B).

Trace Element Analysis. Trace element concentrations in all oyster shell samples were relatively low or below the limits of detection, and no anomalous values were found for any element that could be definitively traced to the DWHOS. Timing of oyster deployment to field sites typically corresponded to an increase in Mg, Sr, Zn, and Ba (data not shown). To focus on elements most likely to indicate oyster exposure to oil hydrocarbons, we opted to show data for the six elements previously suggested to accumulate in sediments or invertebrate shells and tissues due to hydrocarbon pollution [cadmium (Cd), cobalt (Co), molybdenum (Mo), nickel (Ni), lead (Pb), and vanadium(V)].[15,23,24] For most samples the number of detectable elements and average concentration decreased in new growth compared to older growth during (Figure 6A) and after (Figure 6B) the DWHOS spill. The most noticeable difference in trace element composition between during and postspill transplants was the presence of measurable quantities of Pb and V in both old and new shell growth of oysters from the postspill period at Grand Bay and Fort Morgan (compare Figure 6A and B). Pb alone was found in measurable concentrations in new shell growth of postspill oysters from Orange Beach. Vanadium values ranged 0.03–0.08 mg L^{-1} and Pb ranged 0.01–0.02 mg L^{-1}.

■ DISCUSSION

To understand the influence of oil-derived elements on oyster shell, we compared the stable isotope composition in shell of oysters grown before, during, and after the DWHOS with the composition of naturally occurring SPM available as food and with weathered and fresh oil. By making these comparisons, we determined that although oil-derived elements were present in surface and bottom waters where oysters were growing during and after the DWHOS, they did not appear to significantly influence the C and N stable isotope composition in oysters.

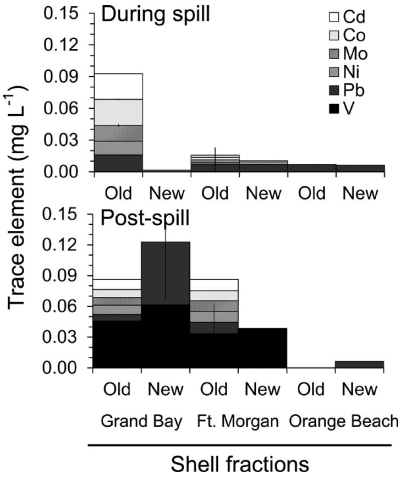

Figure 6. Mean (±SE) concentration of 6 trace elements in shell of oysters transplanted (A) during and (B) after the DWHOS (corresponding to treatments in Table 1). Shell sections were analyzed separately to distinguish elements in old growth (reflecting hatchery and T_0 periods) from those in new growth for each transplant period. Samples for which element concentrations were below the level of detection were not included in mean calculations.

Two major findings support this conclusion. First, there were no shifts in C or N stable isotope ratios in oyster shell relative to the location or timing of surface oil along the Mississippi–Alabama coastline (Figure 4 and SI-Figure S1). Second, stable isotope values were more consistent with SPM-derived diet as opposed to consumption of oil-derived materials (Figures 3 and 5). Based on stable isotope values we measured in fresh and weathered oil, diet comprised primarily of oil-derived substances would be expected to yield stable isotope values of −27 ± 1‰ for C and 3−7‰ for N in oysters. Importantly, δ^{13}C values in oyster shell centered around values typically found in marine algae (∼-21‰), while oil materials, which are derived from terrestrial sources, were significantly lighter (∼-27‰; this study and others[26−28]). Comparison of δ^{15}N ratios between oil and oyster shell demonstrate even more evidently the separation between shell composition and that of oil. The δ^{15}N values we determined in weathered and fresh oil were consistent with previous reports,[26,29,30] and the difference between δ^{15}N‰ in oyster shell and oil far exceeded the expected 2−4‰ fractionation from food source to consumer (Figure 5).[16] These data suggest that oysters either were not exposed to oil hydrocarbons at the sampling locations, did not consume oil materials during the period of study, or consumed too little to be detectable compared to phytoplankton in background SPM, using stable isotope methods.

Data from a parallel study of polycyclic aromatic hydrocarbon (PAH) concentrations in sediment, water, and oysters from sites in Mobile Bay and adjacent waters in early June through mid-November 2010, found higher concentrations of PAHs in oysters at Point aux Pines, AL (our T_0 control site) than at Sand or Denton Reef in Mobile Bay.[31] Total PAH concentrations (tPAH) in oysters ranged from below reporting limits to 522 ng g^{-1} dry weight during and just after the period of active spilling from the MC252 well. This study also found a peak in water tPAHs that roughly corresponded with the estimated timing of oil in the area, and one site in Mobile bay showed possible evidence of DWHOS oil contamination in the water in late June, during the spill. None of the sediment tPAH concentrations in Mobile Bay or adjacent sampled areas exceeded NOAA regulatory SQuiRT guidelines,[32] but concentrations were consistent with values measured for other oil spills. Overall, these data support the notion that the area experienced some oil exposure, but the relatively low measured values in oysters, absence of prespill data, and potential for rapid depuration rates in oysters[33] make it difficult to determine the extent of direct oil contamination of oysters based on PAH data alone.

Given the relatively distinct stable isotopic signature in oil materials compared to expected oyster diet, oil materials should be readily distinguishable if consumed and assimilated into oyster shell and soft tissues. The Mobile Bay system and nearby waters, however, are highly influenced by freshwater discharge.[34,35] As a result, locally available SPM often reflects both the marine and freshwater/terrestrial influences to this system.[28,36] Because of this freshwater influence, in some cases the difference between δ^{13}C values in oil materials and SPM was very small (Figure 3), particularly for the northernmost transplant site in Mobile bay (Denton reef), which is more consistently influenced by freshwater discharge than other transplant locations (Figure 2, SI-Table 1). In this study, the >1‰ difference in δ^{13}C values between oyster shell and bulk SPM at some sites appears to be due to preferential selection and assimilation of the marine phytoplankton component of bulk SPM.[37] Collection of SPM from nearby sites or somewhat different time periods than the period of study may also contribute to variation in δ^{13}C between oysters and SPM. The difference between SPM and oyster shell, however, was not greater when collected at remote sites or times compared to site-specific collection during the transplant period (cf Figure 5). Considering these points, it seems that oysters at transplant sites primarily assimilated elements from local SPM, particularly marine algae, regardless of freshwater inputs or oil-related influences to local waters. In cases where oysters or other species may not be selective, the isotopic similarity between freshwater sources and oil materials could confound detection of elements derived form oil materials. These data highlight the need to consider the distinctiveness of source end points when applying stable isotope data to detect consumption of oil materials by biota in freshwater influenced systems.

Comparison of isotope composition between newly deposited oyster shell and adductor muscle revealed differences in C and N assimilation. In the case of this study, the stable isotope ratios we determined in newly deposited shell reflected the discrete periods of study, before, during, or after the DWHOS, while values in adductor muscle reflected dietary components during the transplant period as well as any elements previously consumed (at T_0 sites) but not yet metabolized or turned-over. Previous studies found turnover times for soft tissues were >120 days for adult shellfish,[37,38] which is longer than the ∼60 day transplant periods for oysters in this study, supporting the notion that adductor muscle represented a mixture of recent and previously assimilated diet components. These observations suggest that during the transplant periods, oyster diet was slightly heavier in δ^{13}C to achieve values near −20‰ in newly deposited shell compared to −23‰ in adductor muscle. A shift toward heavier δ^{13}C values in newly deposited shell is contrary to the shift expected if lighter oil materials were consumed. The observed isotopic differences between shell and soft tissues may also be due to tissue-specific differences in C assimilation. While δ^{15}N values did not differ between shell and soft tissue in this study (SI-Figure S3B), previous studies found that isotopic differences

between shell and soft tissue in bivalves are common for ^{11}N. C values have not been similarly studied. Regardless of the reason for the small tissue-specific differences in δ^{13}C values, both shell and adductor muscle showed values most consistent with a diet comprised primarily of SPM from local sites (Figure 5).

Implications of this work included defining oil byproducts as a potential food source in the local ecosystem, determining the potential for oil to alter coastal food webs, and defining an alternative fate for oil-derived elements in food webs that is independent of or in addition to microbial breakdown. Using *C. virginica* as a benchmark for sedentary primary consumers in the Gulf of Mexico, we found no evidence of assimilation of oil-derived C and N and, therefore, no evidence of an oyster-based conduit to higher trophic levels. This finding, however, is not an indication that oysters were not exposed to oil; rather it implies that oysters either did not consume oil-derived materials or consumed an insufficient quantity to be clearly detectable against the background of their natural food source. Oil-derived C was found to enter the base of the food web at the level of zooplankton, at least initially, during the active spilling of oil from the DWHOS.[28] Oil was apparently available in high enough concentrations in surface waters during the spill to be rapidly encountered and taken up in sufficient quantities by these small organisms to yield detectable isotopic signatures against background. It is possible that larger sedentary primary consumers such as oysters could have lower likelihood of exposure to and uptake of oil materials because they are patchily distributed and likely exposed to relatively weathered and nonhomogenously distributed oil materials that intermittently reach nearshore bottom waters.[6] Oysters at known oil-exposed sites in Louisiana, for example, showed no PAH contamination or apparent biological signs of exposure to oil 6 months after the DWHOS.[39] PAH concentrations, however, may be affected by physiological condition of oysters (which relatively rapidly depurate accumulated PAHs), and previous studies suggest low PAH concentrations do not necessarily indicate a lack of exposure to contaminants.[33,40]

It is also possible that oysters, which typically slow or cease feeding under stress, may have stopped feeding when exposed to oil materials. In this study, oyster growth during the spill period ranged from 0.09 mm d^{-1} (Denton Reef) to 0.29 mm d^{-1} (Grand Bay) (SI-Table S3). Postspill, growth ranged from 0.04 mm d^{-1} (Orange Beach) to 0.17 mm d^{-1} (Grand Bay). These findings follow normal seasonal growth shifts from mid to late summer, but do not show a clear relationship to the estimated spatial or temporal distribution of oil in the area (SI-Figure S1). These findings suggest that oyster feeding and subsequent growth was not significantly affected by potential oil exposure. We cannot, however, rule out possible ephemeral or episodic exposure to oil, which could limit consumption of oil materials and result in little or no noticeable effect on oyster growth.

Trace element analyses were inconclusive to confirm or reject oyster exposure to oil materials, particularly for oysters growing during the postspill period in August and September 2010. Increased concentrations of V and Pb in oyster shells and tissues following the DWHOS have been anecdotally reported.[41] While values were slightly higher in new shell growth during the postspill period, the apparent increase in V and Pb concentrations in both old and new shell growth fractions in this study is potentially confounding. We cannot discount the possibility that random chance associated with variation among individual oysters may account for these results, given the relatively small number of shell samples analyzed and high number of samples with trace element concentrations below the limits of detection. Relationships between trace element accumulation and exposure to oil hydrocarbons merits further study.

Even in small quantities, consumption of oil-derived substances from the DWHOS had potential to feed secondary production, shift elemental composition of food webs, and provide an alternate pathway of oil degradation that requires more attention. Consideration of the distinctiveness of source end points (i.e., oil-derived materials compared to other terrestrial or freshwater inputs) will be important to further apply and interpret stable isotope data to detect consumption of oil materials in freshwater influenced systems. Due to our lack of detailed knowledge regarding when, where, and how different forms of oil materials affected local biota, consideration of the timing (early during the spill period compared to postspill exposure periods) and estimated mode of exposure (surface oil slicks compared to nonhomogenously distributed weathered oil) may be of particular importance to contextualize and interpret effects of the DWHOS on biota among locations in the northern Gulf of Mexico system.

■ ASSOCIATED CONTENT

Supporting Information

Maps of study site locations compared to surface oil distributions reported by NOAA during and after the DWHOS (SI-Figure S1), example of a source oil "fingerprint match" (SI-Figure S2), mean δ^{13}C‰ and δ^{15}N‰ in oyster shell compared to adductor muscle (SI-Figure S3), temperature and salinity measured at transplant sites during the study (SI-Table S1), δ^{13}C and δ^{15}N values determined in weathered and fresh oil forms (SI-Table S2), and oyster shell growth during and after the DWHOS (SI-Table S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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