



# $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the endangered Kemp's ridley sea turtle *Lepidochelys kempii* after the Deepwater Horizon oil spill

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**ABSTRACT:** The *Deepwater Horizon* explosion in April 2010 and subsequent oil spill released  $3.19 \times 10^6$  barrels ( $5.07 \times 10^8$  l) of MC252 crude oil into important foraging areas of the endangered Kemp's ridley sea turtle *Lepidochelys kempii* (*Lk*) in the northern Gulf of Mexico (GoM). We measured  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in scute biopsy samples from 33 *Lk* nesting in Texas during the period 2010 to 2012. Of these, 27 were equipped with satellite transmitters and were tracked to traditional foraging areas in the northern GoM after the spill. Differences in  $\delta^{13}\text{C}$  between the oldest and newest scute layers from 2010 nesters were not significant, but  $\delta^{13}\text{C}$  in the newest layers from 2011 and 2012 nesters was significantly lower compared to 2010.  $\delta^{15}\text{N}$  differences were not statistically significant. Collectively, the stable isotope and tracking data indicate that the lower  $\delta^{13}\text{C}$  values reflect the incorporation of oil rather than changes in diet or foraging area. Discriminant analysis indicated that 51.5% of the turtles sampled had isotope signatures indicating oil exposure. Growth of the *Lk* population slowed in the years following the spill. The involvement of oil exposure in recent population trends is unknown, but long-term effects may not be evident for many years. Our results indicate that C isotope signatures in scutes may be useful biomarkers of sea turtle exposure to oil.

**KEY WORDS:** Sea turtles · Oil spill · Gulf of Mexico · Biomarkers · Stable isotopes · Satellite telemetry · Tracking

## INTRODUCTION

The April 20, 2010 explosion of the *Deepwater Horizon* (DWH) drilling rig at the MC252 well and subsequent 87-d leak released  $3.19 \times 10^6$  barrels ( $5.07 \times 10^8$  l) of oil into the northern Gulf of Mexico (GoM) (NOAA 2016). At its maximum extent, oil covered 112 115 km<sup>2</sup> of the northern GoM, which contami-

nated the coasts of Louisiana, Mississippi, Alabama, and northern Florida, as well as adjacent waters and wetlands (ERMA 2016; Fig. 1). The oil killed large numbers of marine organisms and contaminated important developmental areas for many species (Antonio et al. 2011, Henkel et al. 2102, White et al. 2012, Rozas et al. 2014). Deleterious effects of the spill were immediately evident, but long-term effects remain to

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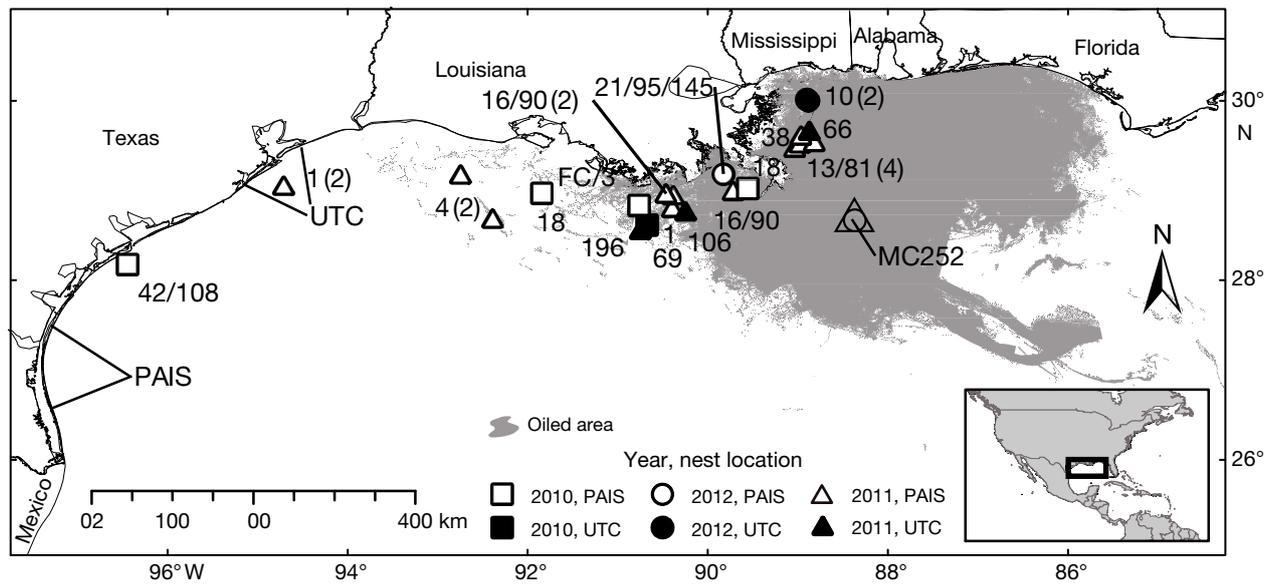


Fig. 1. Location of foraging area centroids in the northern GoM computed from satellite tracking data for Kemp's ridley sea turtles (identified by nest number) that nested in 2 areas of Texas (PAIS: Padre Island National Seashore, open symbols; UTC: upper Texas Coast, solid symbols) during 2010 to 2012. Also shown are the source of the MC252 oil and the maximum extent of the oiled area (gray shading) (ERMA 2016). Numbers in parentheses indicate overlapping centroids of the same turtle; numbers separated by forward slashes are nest numbers for turtles that nested more than once in the indicated year

be determined; organisms surviving initial (acute) impacts of the spill may have sustained permanent or temporary physiological injury, had difficulty finding food, ingested or inhaled oil, consumed oil-contaminated prey, or moved to other foraging areas (e.g. Milton et al. 2003, Moreno et al. 2013, Beyer et al. 2016). Any of these changes could affect the growth rates, reproductive output, overall organism fitness (Peterson et al. 2003) and, depending on the proportion of the population affected, population dynamics of the respective species.

Sea turtles of several species, life stages, and age classes were affected by the spill. Of major concern was the Critically Endangered Kemp's ridley sea turtle *Lepidochelys kempii* (*Lk*), especially considering that most of the turtles known to have been killed were *Lk* (NOAA 2010). The range of *Lk* is generally considered to be the northwestern Atlantic Ocean, but most adults reside in the GoM. Nesting occurs primarily on beaches along the western GoM, from Padre Island, Texas, in the USA southward to Veracruz, México (Pritchard & Márquez 1973). Based on satellite tracking studies, post-nesting *Lk* primarily migrate northeastward and forage in a shallow (approx. 10 to 40 m depth) coastal corridor from Louisiana to southwestern Florida, where there is strong foraging area fidelity (Seney & Landry 2008, Shaver & Rubio 2008, Shaver et al. 2013, 2016a). A 13-yr tracking study of nesting *Lk* tagged at Padre

Island National Seashore (PAIS), Texas, and Rancho Nuevo, Mexico, indicated that waters off Louisiana are consistently important foraging areas (Shaver et al. 2013). Coastal Louisiana also represents foraging habitat for adult female *Lk* that nest along the upper Texas coast (UTC) and for immature turtles (Seney & Landry 2008). These areas were heavily contaminated by oil during and after the spill (Fig. 1), and both direct observation (Wallace et al. 2017) and post-spill modeling of the oil trajectory (Putman et al. 2015) indicated that many *Lk* were exposed.

International conservation efforts implemented since the mid-1970s have resulted in partial recovery of the *Lk* population (Caillouet 2011, Galloway et al. 2016a,b, Shaver et al. 2016b, Wibbels & Bevan 2016). The DWH event began early in the 2010 nesting season. Although substantially greater numbers of *Lk* nest in Mexico than in Texas, nest numbers in Texas and Mexico are highly correlated (Shaver et al. 2016b). Nest numbers in Mexico and Texas during the 2010 nesting season decreased 37% from 2009, as did the number of hatchlings released (Crowder & Heppell 2011). Nest numbers have remained lower than expected in years since the spill (Bevan et al. 2016, Shaver et al. 2016b); however, the extent to which the spill was involved in the recent decline is not clear.

Stable isotopes of carbon (C) and nitrogen (N) incorporated into the scutes of sea turtles provide a

history of foraging and habitat use when serial samples of the scute are analyzed (Reich et al. 2007). Scutes are inert keratinized tissue that grows continuously. New scute is formed from a layer of epidermal tissue that overlies the bony shell of a turtle and produces successive layers 'from the bottom up'. Thus, the oldest dietary record is retained in the outermost (dorsal) layer, and each successive layer (~0.05 mm) reveals more recent diet and habitat use (Reich et al. 2007, 2008, Vander Zanden et al. 2010); however, the amount of time represented by each layer is variable and not precisely known. When used together with satellite tracking, stable isotopes in scute layers can reveal information about foraging history and migration (e.g. Vander Zanden et al. 2016).

Stable isotope (i.e. biomarker) studies completed since 2010 have documented the incorporation of MC252 oil into the tissues of a variety of marine and terrestrial organisms (Mitra et al. 2012, Cherrier et al. 2014, Quintana-Rizzo et al. 2015, Bonisoli-Alquati et al. 2016, Wilson et al. 2016). Therefore, absorption via inhalation, dermal exposure, or direct ingestion of oil by turtles or their prey (Shigenaki 2003), incorporation of oil into the food chain, a change in foraging area, or any combination of these factors after the spill could be reflected in the stable C and (or) N isotope signatures of the most recent (ventral) scute layers. We analyzed stable isotopes of C and N in scute samples obtained from *Lk* females nesting in Texas during 2010, 2011 and 2012, some of which were equipped with satellite transmitters, to initially address 2 questions: (1) Did the tracking data, isotope signatures, or both indicate that the turtles changed their foraging areas or diets after the spill? (2) What proportion of the nesting female population was exposed to oil from the spill based on the isotope signatures? We added a third question, which became apparent during the investigation: (3) Did the isotope signatures indicate that the turtles were exposed to oil or consume prey containing oil (or oil C) after the spill?

## MATERIALS AND METHODS

### Sample collection and analysis

Scute biopsy samples were obtained from 33 *Lk* nesting in Texas during the period 2010 to 2012 at PAIS and on the UTC from Galveston Island to Surfside Beach (Fig. 1; Table 1). Biopsy samples were collected from the posterior and anterior regions of the second costal (lateral) scute with a 6-mm diameter

sterile biopsy punch (Integra Miltex) as described by Reich et al. (2007). Samples were stored in 70% ethanol until preparation for stable isotope analysis. Prior to analysis, each sample was cleaned, rinsed in distilled water, and dried at 60°C for at least 24 h. Lipids were then removed from all samples using an accelerated solvent extractor (Dionex ASE 350, Thermo Fisher Scientific) with petroleum ether as the solvent. The oldest material was collected from the posterior region by grinding with a carbide end mill (Sherline 2010 with 1/16 in SE drill bit) to a depth of 50  $\mu\text{m}$  (yielding ~500  $\mu\text{g}$ ), beginning with the dorsal side of each sample. Successive layers of scute were collected by repeating this procedure on all posterior samples. Anterior scute material was sampled in the same manner except that the layers were collected beginning from the ventral side to capture the most recent foraging history. Samples obtained in this manner, representing the oldest posterior scute layer from the 2010 nesters and the newest material from all 33 turtles (2 or 3 layers per turtle), were analyzed for C and N isotope analysis and used in this study (Table 1). No turtles included in this study were sampled more than once.

Samples were combusted in a Costech ECS 4010 elemental analyzer interfaced via a Finnigan-MAT ConFlow III device to a Finnigan-MAT DeltaPlus XL isotope ratio mass spectrometer in the light stable isotope lab at the University of Florida. Stable isotope abundances were expressed in delta ( $\delta$ ) notation, defined as parts per thousand (‰) relative to the standard as follows:

$$\delta = ([R_{\text{sample}}/R_{\text{standard}}] - 1) / (1000) \quad (1)$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are corresponding ratios of heavy to light isotopes ( $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ ) in the sample and international standard, respectively.  $R_{\text{standard}}$  for  $^{13}\text{C}$  was Vienna Pee Dee Belemnite

Table 1. Number of Kemp's ridley sea turtles nesting at Padre Island National Seashore (PAIS) and on the upper Texas Coast (UTC) during the 2010–2012 nesting seasons from which scute biopsy samples were obtained for stable isotope analysis. Also shown for each year are the numbers of turtles equipped with satellite transmitters, the number of transmitter-equipped turtles for which foraging area centroid locations were computed, and the total number of samples (layers) analyzed.

Nesting season	PAIS	UTC	Total	With transmitters	With centroids	Total samples
2010	10	2	12	7	4	43
2011	8	3	11	10	8	31
2012	7	3	10	10	2	29
Total	25	8	33	27	14	103

(VPDB). The  $R_{\text{standard}}$  for  $^{15}\text{N}$  was atmospheric  $\text{N}_2$ . Internal standards were included in all runs at regular intervals to calibrate the system and assess drift over time.

### Satellite telemetry and statistical analysis

Of the 33 nesting turtles sampled in the period 2010 to 2012, 27 were fitted with platform transmitter terminals as described by Shaver et al. (2013) (Table 1). Location data transmitted to satellites by these terminals were downloaded to and retrieved from [www.seaturtle.org](http://www.seaturtle.org) and used to derive foraging area centroid locations. The centroid locations represent kernel density estimates from the tracks of transmitter-equipped turtles (Shaver et al. 2013 and references therein). The tracking data and associated centroids indicated that all the tracked turtles returned to traditional foraging areas in the northern GoM after nesting, regardless of nesting season or tagging location (Fig. 1).

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the oldest tissue of turtles that nested in 2010, represented by the uppermost posterior scute layer, and the newest tissues of turtles that nested in 2010, 2011 and 2012, represented by the lowermost anterior layers ( $n = 2$  or  $3$ ), were analyzed as a generalized linear mixed-model analysis of variance (ANOVA) using PROC GLIMMIX of the Statistical Analysis System (SAS) Version 9.4 (SAS Institute). In these analyses, scute region–year combinations ( $n = 4$ ) were considered fixed effects and turtles (i.e. subjects;  $n = 33$ ), scute region within turtles ( $n = 1$  or  $2$ ), and layers within scute region (subsamples;  $n = 13$ ) were random effects. The Type-III mean-squares for turtles were used to test the significance of the overall models and of differences between regions and years (singly and in combination). Least-squares means, which are adjusted for all factors in the model and are therefore unbiased with respect to the number of turtles, scute regions, or layers they represent, were compared as single degree of freedom  $F$ -tests. Unless otherwise indicated, a significance level of  $\alpha = 0.05$  was used to judge the results of statistical test.

We also performed a linear discriminant analysis of the stable isotope results together with the tracking data using the R package MASS (Venables & Ripley 2002, R Core Team 2013) to determine the proportion of turtles that nested in 2011 and 2012 that were exposed to oil. We first divided the turtles into 3 main groups: outside the oiled area (OUT), which included only the isotope values representing the oldest posterior scute layer of turtles sampled in 2010, including all 12 individuals, regardless of centroid location or whether centroids were computed (Table 2). We assumed that these turtles had left the spill area prior to April 10 and were therefore not exposed to the oil. The turtles that nested in 2011 and 2012 for which centroids were computed ( $n = 8$  in 2011,  $n = 2$  in 2012) were classified as either within (OIL) or adjacent to (ADJ) the oiled area (Table 2) according to the location of their foraging area centroids ( $n = 1$  to  $4 \text{ ind.}^{-1}$ ) relative to the maximum geographic extent of the oil (Fig. 1). Based on these results, all individuals with centroids except turtle 2011-4 (which was classified as ADJ because it had 2 centroids located outside the oiled area; Fig. 1), were classified as OIL (Table 2). Turtle

Table 2. Kemp's ridley sea turtles nesting in 2 areas of the Texas coast (see Table 1 legend for site abbreviations) during the 2010–2012 nesting seasons that were used in the discriminant analysis to determine proximity to the oil for 2011 and 2012 turtles without tracking data (OUT: outside the oiled area; ADJ: adjacent to the oiled area; OIL: within the oiled area). All 2010 nesters, including those with no transmitter (NA), were assigned to the OUT group regardless of foraging area location. FC: false crawl (turtle crawls onto the beach but does not make a nest)

Nesting season	Nest no.	Nest area	Transmitter no.	Foraging area centroid location	Proximity group
2010	1	PAIS	NA	NA	OUT
2010	6	PAIS	NA	NA	OUT
2010	15	PAIS	47529	Louisiana	OUT
2010	18	PAIS	47562	Texas	OUT
2010	21	PAIS	NA	NA	OUT
2010	22	PAIS	NA	NA	OUT
2010	42	PAIS	47690	NA	OUT
2010	61	UTC	NA	NA	OUT
2010	69	UTC	83245	Louisiana	OUT
2010	104	PAIS	NA	NA	OUT
2010	109	PAIS	NA	NA	OUT
2010	FC	PAIS	47519	Louisiana	OUT
2011	1	PAIS	101136	Texas	OIL
2011	4	PAIS	101137	Louisiana	ADJ
2011	13	PAIS	101138	Mississippi/Louisiana	OIL
2011	16	PAIS	101139	Louisiana	OIL
2011	38	PAIS	106347	Mississippi/Louisiana	OIL
2011	66	UTC	101131	Mississippi/Louisiana	OIL
2011	106	UTC	101132	Louisiana	OIL
2011	196	UTC	101133	Louisiana	OIL
2012	10	UTC	106811	Mississippi/Louisiana	OIL
2012	21	PAIS	112763	Louisiana	OIL

2012-21, which had an early centroid located south of Galveston, Texas, was classified as OIL because it had a later centroid located southeast of Grand Isle, Louisiana, that we assumed to be its primary foraging area (Fig. 1). Differences among the stable isotope data representing the newest anterior layers of the turtles in each group were evaluated with a multivariate analysis of variance (MANOVA), which indicated that differences among the proximity groups were statistically significant ( $F_{2,35} = 4.32$ ,  $p = 0.021$ ). However, a post hoc test indicated that only the isotope values of the OIL and OUT (i.e. 2010) groups differed significantly. The isotope values of turtle 2011-4 (ADJ) were not significantly different from either of the other 2 groups. This individual was therefore excluded from the discriminant analysis because it could not be unambiguously classified as either OIL or OUT. To test the accuracy of the discriminant analysis, we used the isotope values of the OIL and OUT groups as known signatures and conducted a leave-one-out cross-validation test. This test yielded a 76.2% overall accuracy of classification; only 1 (of 9) turtles in the OUT group (all 2010) was misclassified, while 8 (of 12) turtles in the OIL group were correctly classified. After validation, we conducted a second discriminant analysis using the posterior probabilities of the OIL and OUT groups to classify turtles with unknown proximity. The means of the newest anterior layers of each turtle were used in this analysis.

## RESULTS

Raw data (stable isotope results as  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in individual scute layers and foraging area centroids computed from tracking data) are available online (<https://doi.org/10.5066/F70C4SXJ>).

### Stable isotopes in scute layers

Mean  $\delta^{15}\text{N}$  in *Lk* ranged from 10.98 (anterior 2010) to 11.37 (posterior 2010), but there were no statistically significant differences (Fig. 2). Neither the overall ANOVA model ( $F_{3,42} = 0.10$ ,  $p = 0.957$ ) nor any of the contrasts of least-squares means were significant ( $F_{1,42} = <0.01-0.30$ ,  $p = 0.587-0.981$ ). In other words, the oldest and newest tissue layers obtained from turtles that nested in 2010 were identical, and there was no change in the most recent tissues obtained from turtles that nested in 2010, 2011, or 2012.

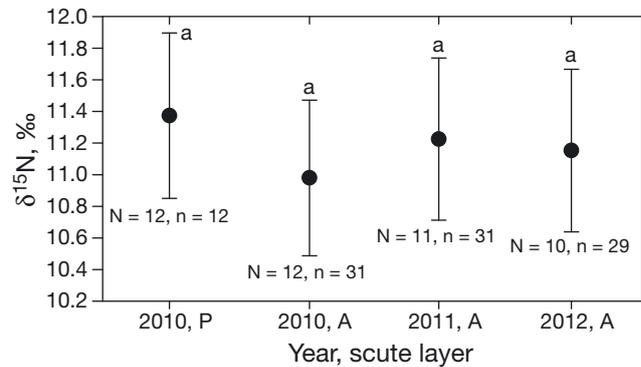


Fig. 2. Least-squares mean  $\delta^{15}\text{N}$  ( $\pm 95\%$  confidence limits) in the oldest (posterior, P) and newest (anterior, A) scute layers from Kemp's ridley sea turtles that nested in 2010 to 2012. Means containing the same letter are not significantly different ( $p < 0.05$ ). N: number of turtles; n: number of samples (layers)

In contrast to  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  differed significantly among years. Mean  $\delta^{13}\text{C}$  ranged from  $-17.2$  (posterior 2010) to  $-18.1$  (anterior 2011) (Fig. 3). The overall ANOVA model was only marginally significant ( $F_{3,42} = 2.26$ ,  $p = 0.095$ ), and the oldest (posterior) and newest (anterior) scute tissues from turtles that nested in 2010 were not significantly different ( $F_{1,42} = 0.37$ ,  $p = 0.545$ ). In contrast, the oldest tissue from the 2010 nesters differed significantly from the newest tissues of the 2011 nesters ( $p = 0.025$ ; Fig. 3), as did the oldest and newest tissues from the 2010 nesters considered together ( $p = 0.019$ ). The newest (anterior) tissues of 2010 nesters were not significantly different from those of 2012 nesters ( $F_{1,42} = 1.30$ ,  $p = 0.2604$ ), but the

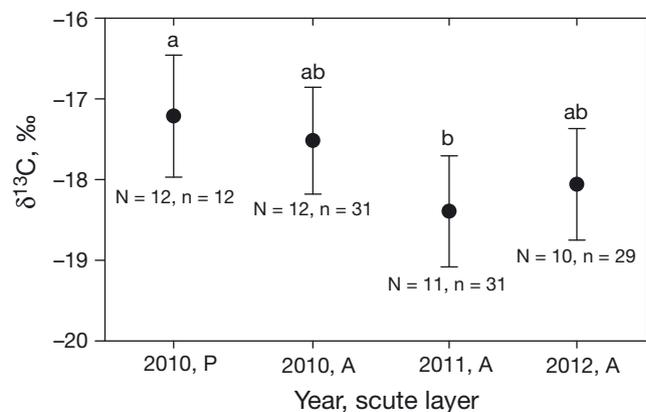


Fig. 3. Least-squares mean  $\delta^{13}\text{C}$  ( $\pm 95\%$  confidence limits) in the oldest (posterior, P) and newest (anterior, A) scute layers from Kemp's ridley sea turtles that nested in 2010 to 2012. Means containing the same letter are not significantly different ( $p < 0.05$ ). The 2010 values considered together (A and P) also differed significantly from 2011 ( $p = 0.019$ ) but not from 2012 ( $p = 0.109$ ). N: number of turtles; n: number of samples (layers)

difference between the newest 2010 and 2011 tissues approached significance ( $F_{1,42} = 3.42$ ,  $p = 0.071$ ; Fig. 3). The newest tissues from the 2011 and 2012 nesters considered together also differed significantly from the oldest and newest tissue of the 2010 nesters considered together ( $F_{1,42} = 6.15$ ,  $p = 0.017$ ); however, the difference between the oldest and newest tissue from the 2010 nesters and the newest tissue from the 2012 nesters was only marginally significant ( $F_{1,42} = 2.68$ ,  $p = 0.109$ ). Overall, there was a statistically significant  $\delta^{13}\text{C}$  decrease ( $\Delta^{13}\text{C}$ ) of about 1.0 ‰ from 2010 to 2011 followed by an increase of about 0.35 ‰ from 2011 to 2012 that was not significant (Fig. 3).

#### Assignment of untracked turtles to proximity groups

The discriminant analysis assigned 15 of the 23 untracked turtles to the OUT group (65%) and the other 8 (35%) to the OIL group based on their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures (Fig. 4). The probability of correct classification ranged from 0.505 to 0.997, and was  $>0.75$  for 11 of the 23 turtles (61%). When separated by year, 8 of the 12 turtles sampled in 2010 were assigned to the OUT group (67%); 4 were assigned to the OIL group (33%). Three of the 4 incor-

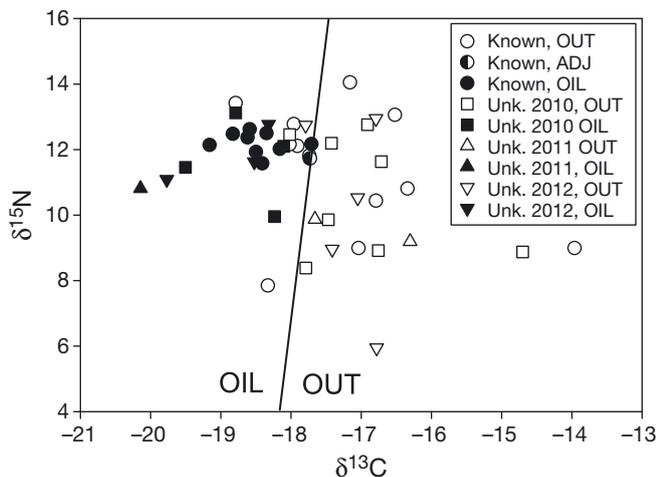


Fig. 4. Assignment of Kemp's ridley sea turtles sampled in the GoM in 2011 and 2012 with unknown (Unk.) foraging areas to proximity groups based on the isotope values of turtles with known foraging areas, defined as outside (OUT), adjacent (ADJ) or oiled (OIL) with respect to the DWH oil spill, by linear discriminant analysis. The OUT group includes all 2010 turtles, which were presumed to have left the oiled area before the spill. The diagonal line represents the OIL vs. OUT decision boundary computed from the function  $y(x) = w^T x + w_0$ , where  $w^T$  is the weight vector (discriminant functions),  $w_0$  is the bias or threshold weight,  $x$  is the predictor variable, and  $y(x)$  is the response variable

rectly assigned turtles were near the decision boundary (Fig. 4). Of the unknown turtles sampled in 2011 (1 untagged, 2 without centroids), 2 were assigned to the OUT group (67%) and one to the OIL group (33.3%); and of the 8 turtles from 2012 without centroids, 5 were assigned to the OUT group (63%) and 3 to the OIL group (37%). Considering all turtles sampled ( $n = 33$ ), the estimated proportion of nesting turtles potentially exposed to the oil was 51.5% (9 with foraging area centroids in the oiled area and 8 assigned to the oil group by discriminant analysis).

## DISCUSSION

#### Foraging and migratory history indicated by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

Foraging history inference through stable isotope analysis depends on the residence time of C and N isotopes in different tissue types and, in our study, scute layers (Reich et al. 2008). A dietary study of an adult female *Lk* held in captivity for 1 yr documented the C and N isotope change that occurred during a wild-to-captive diet shift; stable isotopes of C and N signatures of the wild diet were retained in posterior scute through the 1-yr study period and thus had a residence time of at least 12 mo (Iseton & Reich 2013). Isotope residence times increase with increasing body mass and decline with decreasing growth rates (Martínez del Río et al. 2009). Because the studied turtle was maintained on a protein-rich diet, we assume that it was in an accelerated growth state and are therefore confident that under normal conditions, the average residence time is longer than our sample period (1 yr) and that our samples are appropriate for assessing stable isotope signatures of turtles prior to their migration to the nesting grounds.

We assumed that the 2010 nesters had left the oiled area either prior to the beginning of the spill or before extensive contamination of their foraging area in the northern GoM. Based on this assumption, we used the 2010 samples as a pre-spill baseline for both C and N. Our data indicate that  $\delta^{13}\text{C}$  declined after the spill (Fig. 3). Conversely, there was no change in  $\delta^{15}\text{N}$  (Fig. 2). The  $\delta^{15}\text{N}$  results indicate that the *Lk* diet (preferred prey) did not change from 2010 to 2012 or that the turtles at least fed at the same or similar trophic level. Although the stable isotope results could be interpreted as indicating that *Lk* moved from their main foraging area in the northern GoM to secondary areas in the southern or eastern GoM (Shaver et al. 2013), we consider this unlikely for the following reasons: (1)

A study of loggerhead sea turtles *Caretta caretta* that used stable C and N isotopes to create isoscapes of foraging areas in the GoM showed that  $\delta^{13}\text{C}$  values in the 3 regions used by both species (northern, eastern and southern GoM) are not significantly different and therefore could not be differentiated (Vander Zanden et al. 2015). (2) Vander Zanden et al. (2015) also reported significant differences in  $\delta^{15}\text{N}$  between the northern GoM and the eastern and southern GoM. If the *Lk* had moved to the southern or eastern GoM, we would have detected a significant change in  $\delta^{15}\text{N}$  between years, which we did not. Gelpi et al. (2013) also documented an inshore–offshore  $\delta^{15}\text{N}$  increase in blue crabs *Callinectes sapidus* along the Louisiana coast. Blue crabs are important in the *Lk* diet (Bjorndal 1997 and references therein). Consequently, the absence of any  $\delta^{15}\text{N}$  difference indicates that the turtles did not forage further offshore, which is also supported by the tracking data (Fig. 1). (3) Long-term satellite tracking has shown that many nesting *Lk* females (98%,  $n = 24$  over 13 yr) travel to foraging areas in the northern GoM and mainly concentrate in coastal waters off Louisiana, Mississippi and Alabama (Shaver & Rubio 2008, Shaver et al. 2013, 2016a). Gelpi et al. (2013) also documented an east–west  $\delta^{13}\text{C}$  decline in blue crabs along the Louisiana coast; nevertheless, our satellite tracking data indicate that post-nesting *Lk* returned to traditional foraging areas in the northern GoM during all 3 nesting seasons, and there was no indication of a westward shift that would result in a  $\delta^{13}\text{C}$  change (Fig. 1).

### Incorporation of oil C

Our findings indicate that the  $\delta^{13}\text{C}$  change we detected resulted from the incorporation of oil and gas C and not from a post-spill foraging area or diet shift. Our results differ from those of Vander Zanden et al. (2016), who found no  $\delta^{13}\text{C}$  change in the scutes of loggerhead turtles that foraged in the oiled area after the spill;  $\delta^{13}\text{C}$  in the loggerheads was ca.  $-15$  to  $-18$  ‰ before and after the spill, which is similar to our pre-spill values for *Lk* (Fig. 3). The  $\delta^{15}\text{N}$  values were similar (ca. 10 to 15 ‰) in both species and did not change over time (Vander Zanden et al. 2016; Fig. 2). Although the diets of *C. caretta* and *Lk* overlap (Bjorndal 1997 and references therein), the differing  $\delta^{13}\text{C}$  results may reflect diet differences between the species.

Our results, together with previous satellite tracking data (Shaver et al. 2013, 2016a), indicate that the principal *Lk* foraging grounds in the northern GoM were

contaminated by MC252 oil after the DWH spill in April 2010, but that *Lk* continued to forage in these areas in 2010 and later years. Loggerhead turtles also returned to the oiled area after the spill (Vander Zanden et al. 2016), but there was no  $\delta^{13}\text{C}$  change indicative of oil exposure in the loggerheads. In contrast, the  $\delta^{13}\text{C}$  change from 2010 to 2011–12 in *Lk* is consistent with the incorporation and subsequent fractionation of C from the oil ( $\delta^{13}\text{C} = -27 \pm 0.2$  ‰; Carmichael et al. 2012) via direct oil ingestion, inhalation, or dermal absorption, the consumption of oil-contaminated prey, or incorporation of oil C at lower levels of the food chain (Shigenaka 2003, Graham et al. 2010) and not a change in foraging location. Other studies that have documented the incorporation of oil C into lower trophic levels and at different depths in the GoM also reported depleted C signatures (Graham et al. 2010, Mitra et al. 2012). In contrast to findings for loggerhead turtles (Vander Zanden et al. 2016), our results indicate that the oil signature was transferred to *Lk*, as also reported for 2 species of mesopelagic GoM fishes (Quintana-Rizzo et al. 2015). In the fish, a  $\delta^{13}\text{C}$  decrease in muscle samples collected 5 mo after the spill relative to pre-spill samples was attributed to the ingestion of oil-contaminated prey (Quintana-Rizzo et al. 2015). Depleted C signatures attributed to the incorporation of MC252 oil have also been reported in fish, invertebrates, and birds inhabiting coastal Louisiana (Fry & Anderson 2014, Bonisoli-Alquati et al. 2016, Wilson et al. 2016). The  $\delta^{13}\text{C}$  change in all of these taxa except the birds (seaside sparrow *Ammodramus maritimus*) was smaller than the  $\sim 1$  ‰ mean difference we detected between the newest scute layers of *Lk* sampled before and after the spill. A larger difference ( $\sim 2.5$  ‰) was reported between the feathers of seaside sparrows foraging in oiled and un-oiled marsh areas of Louisiana after the spill (Bonisoli-Alquati et al. 2016).

The integration of satellite telemetry and stable isotope data (Vander Zanden et al. 2015, 2016) allowed us to determine the isotope signature of turtles from the oiled area, which we used to estimate the proportion of nesting turtles for which centroids were not available that were exposed to the oil in the northern GoM. This was possible because, despite the small number of tracked turtles ( $n = 10$ ) for which centroids were available, the isotope signatures of the turtles that foraged in the oiled area differed significantly from the baseline isotope signature of the oldest scute tissue of turtles sampled in 2010. We established a probability threshold of 0.75 for the assignment of individuals to one of these 2 areas. Although only 11 of the 23 turtles met the 0.75 threshold, the rest did not have probabilities lower

than 0.505. Most of these turtles were close to the decision boundary computed based on the scaled discriminant functions and the mean and prior probabilities of the known groups (Fig. 4).

According to NOAA (2010), 473 oiled turtles were stranded and (or) captured as a result of direct interaction with the oil; 75% were *Lk*. Of these, only 17 died because of oil-related injuries; 456 were rehabilitated and released. Nevertheless, our data indicate that a large percentage of the *Lk* population in the northern GoM showed evidence of oil exposure through 2012, when C signatures in the newest scute tissue of nesting turtles (most recent foraging history) remained depleted in  $\delta^{13}\text{C}$  compared to 2010 values. Evaluation of samples obtained in subsequent years will be important to determine if  $\delta^{13}\text{C}$  returns to pre-spill values in the main *Lk* foraging grounds of the northern GoM.

## SUMMARY AND CONCLUSIONS

The data from the tracked turtles together with those assigned to the oiled area via discriminant analysis indicate that 51.5% of the *Lk* in the northern GoM may have been exposed to the oil. Although post-spill effects of oil on sea turtle reproduction have not been directly documented, oil contamination of the principal *Lk* foraging areas in the northern GoM may also be at least partly involved in the discontinuation of the exponential *Lk* population increase that was evident prior to 2010 (Crowder & Heppell 2011, Shaver et al. 2016b). Bonisoli-Alquati et al. (2016) documented the post-spill incorporation of oil C into seaside sparrow nesting in Louisiana and suggested a linkage between oil exposure and reduced post-spill reproductive success. In contrast to the much shorter-lived sparrows, immature sea turtles exposed to oil and the progeny of exposed adults will not mature for many years. It will also take many years for the large amount of oil and gas C released to the northern GoM to flow through and out of the Gulf ecosystem. Continued evaluation of data from *Lk* nesting beaches (i.e. nest numbers, remigration intervals) will therefore be required to ascertain whether oil contamination of the foraging grounds affects reproductive success and, ultimately, recovery. Growth rate of nesters, remigration intervals, and integration of neophytes into the population, for which there has been recent evidence of change (e.g. Shaver et al. 2016a), should also be evaluated. And finally, our results indicate that C isotope signatures in scutes represent a useful biomarker of oil exposure in sea turtles, which would further

benefit from the analysis of  $^{14}\text{C}$  due to its low abundance in oil (e.g. Chanton et al. 2012, Fry & Anderson 2014, Wilson et al. 2016).

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