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The Status of Louisiana's Diamondback Terrapin (*Malaclemys terrapin*) Populations in the Wake of the *Deepwater Horizon* Oil Spill: Insights from Population Genetic and Contaminant Analyses

Danielle H. Drabeck,[1] Matthew W. H. Chatfield, and Corinne L. Richards-Zawacki

Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, Louisiana 70118 USA

Abstract.—**We studied the population-genetic structure of the Diamondback Terrapin (*Malaclemys terrapin pileata*) in Louisiana and used data from previous studies to compare our results with rangewide patterns of genetic diversity. We analyzed blood plasma to examine the contaminant load in Louisiana terrapins in the wake of the 2010 *Deepwater Horizon* oil spill. Using 16 polymorphic microsatellite loci, we tested for genetic structure to infer site fidelity, connectivity, and gene flow among terrapins in Louisiana estuaries. We found no evidence for population structure within Louisiana, even among sites up to 120 km apart. Comparing our Louisiana samples to data from previous studies using four loci, we found relatively low genetic diversity in Louisiana and other Gulf Coast terrapins. Based on their genetic similarity, our results support previous recommendations that two Gulf Coast subspecies be grouped into one management unit. The low genetic diversity we observed in Gulf Coast populations may be attributable to anthropogenic pressures, including massive overharvesting events in the early 20th century, from which populations have never fully recovered. Chemical analysis of plasma revealed low concentrations of contaminants, although analyses of other tissue types might have yielded a better estimate of oil contaminant sequestration for this species. Although our observed contaminant levels align roughly with levels of oiling at sampled sites, background levels as well as further analysis using more appropriate matrices (liver or fat) are needed to more accurately assess the impact of the 2010 *Deepwater Horizon* oil spill on the health of Gulf Coast terrapin populations.**

The Diamondback Terrapin (*Malaclemys terrapin*) is the only endemic North American turtle that inhabits saltwater and brackish environments (Ernst et al., 1994; Hauswaldt and Glenn, 2005; Brennessel, 2006). Its range extends along the coastline of the eastern United States from Cape Cod, Massachusetts, to southern Texas (Ernst et al., 1994; Hauswaldt and Glenn, 2005). It has been described as a keystone species, important in regulating prey populations (e.g., the Periwinkle Snail, *Littorina irrorata*), with a diet that includes fish, Blue Crabs (*Callinectes sapidus*), clams, and mollusks (Tucker et al., 1995; Leveque, 2000; Brennessel, 2006; Erickson et al., 2011).

Malaclemys terrapin has seven recognized subspecies, one of which (*M. terrapin terrapin*) is listed as endangered in Rhode Island and threatened in Massachusetts (Ernst et al., 1994; Hart dissertation, 2005). Some of the subspecies that occur from the Chesapeake Bay south to the Gulf Coast have been given "special concern" status by the states in which they occur. This includes *Malaclemys terrapin pileata*, the subspecies found in Louisiana and Texas. In the first study of the genetic structure of *M. terrapin*, Lamb and Avise (1992) were only able to group Gulf Coast populations against Atlantic coast populations (from Cape Canaveral northward) by a single mitochondrial DNA (mtDNA) restriction site polymorphism. Based on microsatellite analyses, Hart (2005) more recently recommended a regrouping of subspecies into 6 management units (MUs), distinguishable by statistically significant genetic breaks, and proposed that the three Gulf Coast subspecies (*M. t. pileata, Malaclemys terrapin littoralis, Malaclemys terrapin macrospilota*) be grouped as one MU.

The greatest documented threats to terrapin populations include mortality from drowning in crab traps, road mortality, loss of habitat, boat propeller injuries, and habitat contamination by environmental pollutants (Bishop, 1983; Wood and Herlands, 1997; Roosenburg et al., 1997, 1999; Hoyle and Gibbons, 2000; Gibbons et al., 2001; Tucker et al., 2001; Holliday et al., 2008; Basile et al., 2011). The current status of terrapin populations in Louisiana is unknown (Seigel and Gibbons, 1995), in large part because of a paucity of published studies. Louisiana terrapins were harvested to near extinction for sale as a food item in the early 20th century (Coker, 1906, 1931). The traffic in terrapins reduced populations so much that prices in Savannah, Georgia reached as high as $90 per dozen in the 1920s (Dundee and Rossman, 1989). In response to this sudden decline, both government and private terrapin farms were set up to provide a consistent source of terrapin meat (Coker, 1906, 1920; Barney, 1922; Hildebrand and Hatsel, 1926; Hildebrand, 1928). Some small-scale private terrapin farming, as well as harvesting of wild turtles for food, still occurs in parts of southern Louisiana (pers. comm. to authors from local fisherman). The demand for terrapin meat has not been a major concern for the species since the 1930s. However, the sharp decline in some terrapin populations in the early part of the 20th century, as well as the massive translocation of terrapins to and from the Gulf Coast to markets, farms, and for wild release (Coker, 1920; Hildebrand and Hatsel, 1926; Hauswaldt and Glenn, 2005), has likely impacted the genetic structure and variability of the species. In addition to these documented impacts on terrapins across their range, there are likely impacts from less studied threats such as rapid habitat loss resulting from hurricanes and environmental contaminants from oil spills.

Research on the toxic effects of oil on animals has focused mainly on aromatic compounds, including polycyclic aromatic hydrocarbons (PAHs) and other chemicals present in crude oil, after major spill events. In birds, PAHs have been shown to cause acute effects such as thyroid hypertrophy, weight loss, gut damage, renal damage, liver damage, increased mortality, and immune suppression among other toxic effects (Troisi et al., 2006). Studies of the chronic effects of PAH contamination on other vertebrates after a major oiling event have shown evidence of sequestration through food chains and maternal transfer, as well as long-term effects such as DNA damage,

[1]Corresponding Author. Present address: Department of Ecology, Evolution, and Behavior, University of Minnesota, Minnesota 55108 USA. E-mail: Danielle.Drabeck@gmail.com
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cancer, and reproductive failure (Hall and Oris, 1991; Alam and Brim, 2000; Martineau et al., 2002; Roy et al., 2003; Brown and Steinert, 2004; Lemiere et al., 2005; Seruto et al., 2005; Troisi and Borjesson, 2005; Troisi et al., 2006; Holliday et al., 2008; Kannan and Perrotta, 2008; Moon et al., 2011). Chemical contamination can also affect the genetic variability of populations in a number of ways, such as inducing heritable mutations, altering the selective landscape, or causing population bottlenecks (Bickham et al., 2000). These effects are predicted to be especially important in small, isolated populations where the outcome of contamination may range from reduced fitness to local extinction.

The Diamondback Terrapin is a good indicator species for long-term studies of contamination and bioaccumulation in an estuarine ecosystem because it is long-lived (up to 30 yr), a top predator, and has a broad distribution (Brennessel, 2006). Terrapins have also been shown to display site fidelity for nesting beaches, creeks, and streams. This has raised concern about increased vulnerability to local extirpations while also making *M. terrapin* an indicator species in assessing local effects of environmental contaminants (Roosenburg, 1994; Gibbons et al., 2001; Holliday et al., 2008; Basile et al., 2011). For all these reasons, the Diamondback Terrapin, along with other turtle species, has been used as an indicator to detect persistent organic pollutants (POPs), PAHs, mercury, and estrogenic PCB effects in estuaries (Bergeron et al., 1994; Kannan et al., 1998; Alam and Brim, 2000; Blanvillian et al., 2007; Basile et al., 2011). Recently, Diamondback Terrapin eggs were found to contain PAH concentrations exceeding those associated with egg failure in Loggerhead Sea Turtles (*Caretta caretta*) (Alam and Brim, 2000; Holliday et al., 2008). Although egg contaminant levels varied among sites, they were not correlated with the physical presence of oil on nesting beaches (Holliday et al., 2008). This suggests that a more likely route of exposure may be through maternal transfer, where PAHs bind with lipid-rich vitellogenin and are later incorporated into developing follicles. A recent study has shown that blood plasma is a good indicator of POP loads in *M. terrapin* body fat and is minimally invasive to collect and that the presence of POPs in plasma may be predictive of maternal transfer in females (Basile et al., 2011). However, this method has been used only once previously to successfully detect PAHs in reptiles (Camacho et al., 2012).

In this study, we examine the population genetics of Louisiana Diamondback Terrapin populations and place our findings in the context of the diversity found in populations sampled previously from across the species' range. Our work aims to provide information about the size, connectivity, and health of Louisiana populations, including measures of contaminant exposure following the *Deepwater Horizon* oil spill, which occurred off the Louisiana coast in April 2010. We predicted that Louisiana terrapins would show genetic similarity to East Coast terrapins, as has been demonstrated in previous studies of *M. terrapin* populations from Texas. Also, we predicted that, in comparison with the rest of the species' range, Louisiana terrapin populations would deviate from a pattern of isolation by distance (IBD) as a result of massive translocation events to and from the East Coast and releases from closed terrapin farms in the 1930s (Coker, 1920; Hauswaldt and Glenn, 2005). Louisiana terrapins were also predicted to show evidence of population bottlenecks as a result of overhunting events in the middle part of the 20th century, in the 2010 *Deepwater Horizon* oil spill, or in both (Hildebrand and Hatsel, 1926; Hauswaldt and Glenn, 2005). For our chemical analyses, we predicted that we would find oil-derived contaminants to be present at detectable levels in the plasma of terrapins collected after the oil spill but at lower concentrations than what has been seen in eggs. Based on the NOAA/EPA Environmental Response Management Application Gulf Response (ERMA) report, which provides geographic data on beach and wetland oiling, as well as observations made during our fieldwork, we predicted that terrapins from our Cocodrie site would show higher levels of PAHs and other oil-derived contaminants in their blood plasma than would terrapins from more protected sites in Lake Machias and Shell Beach (for ERMA maps of oiling and marine animal stranding, see Appendix 1, Supplementary Figs. S4 and S5. Appendix, tables, and figures are available at: http://dx.doi.org/10.1670/12-186s1).

Materials and Methods

Study Populations.—Thirty-one *M. terrapin* were sampled for genetic material from four localities along southeastern Louisiana salt marsh and coastal watersheds between August and December 2010: Cocodrie and surrounding watersheds (Cx, $N = 7$), Shell Beach near the Mississippi River Gulf Outlet (MRGO) Waterway (SBx, $N = 10$), Lake Machias (LMx, $N = 8$), and Port Fourchon (PFx, $N = 4$) (Fig. 1). Two additional samples (MUSE) were obtained from the Genetic Resources Department at the Louisiana State University Museum of Natural Science. These were collected in September of 1996 in Violet, Louisiana, near the Shell Beach/Mississippi River Gulf Outlet (MRGO) waterway (Fig. 1). The greatest Euclidian distance between any two sites is 120 km. Several capture methods for this species that have been successful in other parts of the country, including gill nets, circular turtle traps, funnel traps, drift fences, dip nets, hand capture, and modified crab pots (Roosenburg et al., 2003; Sheridan et al., 2010; Butler et al., 2012; Selman and Baccigalopi, 2012), were tried but failed to capture terrapins. This could have been attributable to our sampling in the dormant season, the presence of only a small number of terrapins over a very large and difficult to access marsh landscape, or both. The only method that provided any success was collaboration with local fisherman to collect live by-catch from shrimp trawling nets. Although our network of cooperative fishing companies spanned the Louisiana Gulf Coast, only 29 terrapins were captured over a period of 4 months in this or any manner. Genetic data for an additional 318 *M. terrapin* across 7 states (Texas, Florida, South Carolina, North Carolina, Maryland, New Jersey, and New York) from Hauswaldt (2004) were used for comparison with the Louisiana samples. Because Hauswaldt (2004) found no genetic structure among 171 samples from South Carolina, we grouped these samples as one population representing the Charleston harbor area (the center point of sampled South Carolina localities). In addition to this dataset, we used summary statistics from Hart's (2005) range-wide terrapin study for comparison against our new and re-analyzed data.

Microsatellite Genotyping.—Genomic DNA was isolated from blood (Cx, LMx, SBx), tail tip (PFx), and archived tissues (MUSE) using a QIAGEN DNeasy kit according to the standard protocols for blood and animal tissue. Genomic DNA was also extracted from blood samples from 10 of the terrapins in the Hauswaldt (2004) study to insure consistency in allele calling across datasets. For the Louisiana samples, DNA was amplified at 4 microsatellite loci developed for *M. terrapin* (Hauswaldt and Glenn, 2003) and 12 microsatellite loci developed for *Glyptemys muhlenbergii* (King

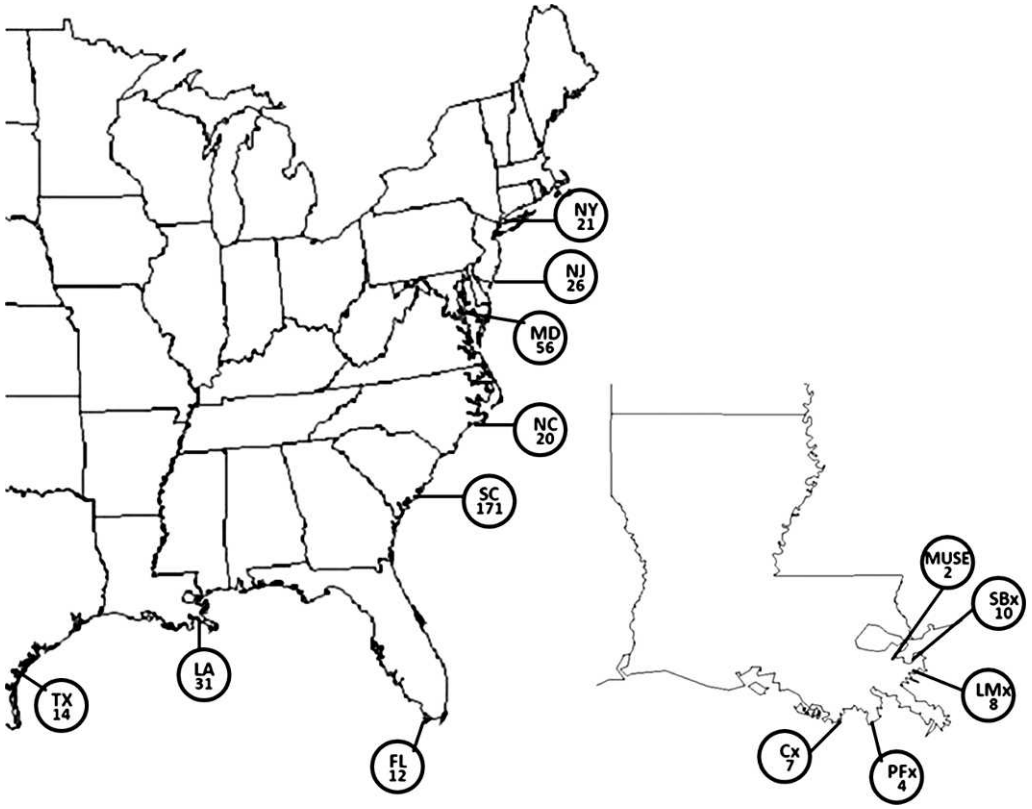

FIG. 1. Sample sizes and localities for *Malaclemys terrapin* included in this study. Samples from Louisiana were collected in 2010 along with 2 museum samples collected in 1996, whereas data from other states are from Hauswaldt (2004).

and Jullian, 2004) yielding 16 microsatellite loci. Forward primers were labeled with a 5′-fluorescent tag (HEX, NED, or 6-FAM). Polymerase chain reactions (PCRs) were carried out in 10-uL volumes containing 1× Ampli*Taq* Gold Master Mix (Applied Biosystems), 10–50 ηg genomic DNA, and 0.5 μM each of forward and reverse primers. Each locus was amplified individually on an MBS Satellite 0.2G Thermocycler (Thermo Electron Corporation) using an initial denaturation step at 94°C for 2 min. This was followed by 35 cycles of 94°C for 45 sec, annealing at 56°C for 45 sec, and extension at 72°C for 2 min. The final extension step was 72°C for 10 min. Amplified PCR products were run on an ABI 3100 DNA Analyzer (Applied Biosystems). Fragments were sized with ROX-500 size standard (BioVentures, Inc., Murfreesboro, TN) and collected with GeneScan v3.1 (Applied Biosystems). Scoring was performed with GeneMarker 1.85 (SoftGenetics LLC, State College, PA). Summary statistics were calculated using GENALEX v6.1 (Peakall and Smouse, 2006) and MSA 4.0 (Dieringer and Schlötterer, 2002).

Statistical Analysis.—All analyses of genetic patterns within Louisiana and comparisons of summary statistics with Hart (2005) included all 16 microsatellite loci. However, for comparative analyses with reanalyzed data from Hauswaldt (2004), only the 4 loci shared between our two datasets were used. Allelic richness, as well as a measure of distance determined by proportion of shared alleles, was calculated using MSA 4.0 (Dieringer and Schlötterer, 2002). Tests of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were performed with GENEPOP 4.0 (Raymond and Rousset, 1995). We tested for correlations between pairs of measures, as well as between measures of genetic distance and geographic distance using a Mantel test in GENALEX version 6.1 with 9,999 permutations (Peakall and Smouse, 2006).

Analyses of molecular variance (AMOVAs; Excoffier et al., 1992), pairwise-values (90,000 permutations), and measures of population subdivision (global-values), were estimated in ARLEQUIN version 3.5 (Wier and Cockerham, 1984; Schneider et al., 2000). Although our analysis indicated that TerpSH8 conformed to HWE expectations, Hauswaldt (2004) did detect divergence from HWE at this locus. Therefore, we ran all AMOVA analyses with and without this locus and two others that we found to depart from HWE (D121, B91). AMOVAs were performed to test the null hypothesis of no population structure, as well as several more specific hypotheses of regional population structure (following Hart, 2005; Hauswaldt and Glenn, 2005). Pairwise chord distances D_{CE} (Cavalli-Sforza and Edwards, 1967) were calculated and fit to an unrooted phylogram using the neighbor-joining algorithm in TREEFIT 1.2 (Kalinowski, 2009). Support for inner branch lengths were calculated by bootstrapping over loci in the same program for 999,999 iterations (Fig. 2).

A test to detect significant heterozygote excess or deficiency for our Louisiana samples was run with BOTTLENECK 1.2.02 using a Wilcoxon sign-rank test (Luikart et al., 1997). The program was run using both the Infinite Alleles Model (IAM; Maruyama and Fuerst, 1985) and the Stepwise Mutation Model (SMM; Cornuet and Luikart, 1997). The ratio of number of alleles per locus to range of allele sizes by number of repeats (M) was also calculated to detect recent population bottlenecks (Garza and Williamson, 2001). Garza and Williamson (2001) predicted that a loss of alleles over the range of allele-size would result in a smaller ratio, as the range would stay relatively constant, whereas alleles within the range were lost as long as

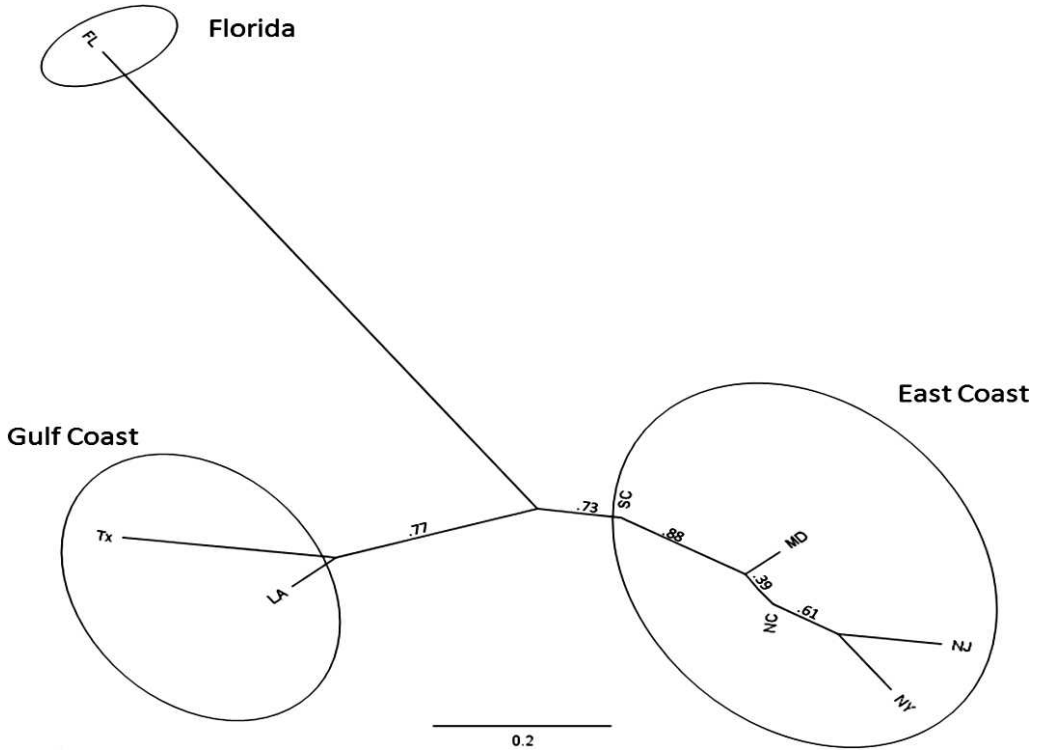

FIG. 2. Unrooted neighbor-joining tree of pairwise genetic distances measured by chord distance D_{CE} (Cavalli-Sforza and Edwards, 1967). Circles indicate regional groupings inserted after visual inspection of branch lengths. East Coast terrapins were grouped as one region because the purpose of this study was not to reorganize regional groupings outside of our sampling area (for further resolution of the east coast region, see Hart [2005] and Hauswaldt [2004]). Numbers on branches indicate bootstrap values for 999,999 iterations.

the largest and smallest alleles were not lost most often (Garza and Williamson, 2001). Tests for sex-biased dispersal in Louisiana were conducted via the method of Goudet (2002) with FSTAT (vers. 2.9.3.2). The statistical descriptors used were, Mean Assignment Indices (mAIc), and Assignment variance (vAIc) as in Hart (2005).

Assuming a priori the population structure found in Hart (2005), Hauswaldt (2004), and Hauswaldt and Glen (2005), Bayesian assignment tests were performed to assign individuals from the more recently sampled Louisiana populations to those sampled across the species range by Hauswaldt (2004) using GENECLASS 2.0 (Rannala and Mountain, 1997; Piry et al., 2004). This analysis uses three criteria for likelihood estimation (genetic distance, allele frequency, and Bayesian criteria) to test for recent migrants and estimate reliability of assignment to regional groupings. The test was run by the exclusion method, in which Louisiana terrapins were treated as individuals of unknown origin and either excluded ($P < 0.20$) or assigned ($P > 0.20$) to sampled sites from across the species' range (Beneteau et al., 2012). Probabilities of assignment were calculated using 10,000 simulated individuals (Paetkau et al., 2004).

Chemical Analysis.—Blood (0.95–6.5 mL per animal via the dorsal cervical sinus, which was 5–8% of total blood volume estimated by weight (HACC, 2004; see Appendix 1, Table S5 online: available at dx.doi.org/10.1670/12-186s1) was extracted from terrapins from three Louisiana localities between September and November 2010. Animals were not sacrificed for liver or fat tissue because of their uncertain conservation status. Both syringe and blood vials were coated in 8% sodium heparin immediately before blood was extracted. Blood was then stored in glass vials and chilled or immediately separated via centrifugation. Clear plasma was extracted from the supernatant and stored at −20°C until thawed for further analysis. Because of the low volume of plasma extracted from each individual, samples were pooled by site (Cocodrie, Shell Beach, and Lake Machias) and sent on ice to Columbia Analytical Services (Kelso, WA) for gas chromatography/mass spectrometry (GC/MS) analysis. With this analysis, we sought to determine the presence and quantity of polycyclic aromatic hydrocarbons (PAHs) and other aromatic compounds associated with petroleum contamination from a standard panel of 27 chemicals included in EPA method 8270D for semivolatile organic compounds (Appendix 1, Supplementary Table 6, online). This panel was chosen because it provided a large degree of overlap with previous work done to detect PAHs in terrapin eggs and plasma from Loggerhead Sea Turtles (Holliday et al., 2008; Camacho et al., 2012). Automated Soxhlet extraction in 1 : 1 (*v*/*v*) acetone : hexane (EPA method 3541) was used as it has been shown to be appropriate for semi-volatile organic compounds (Stewart, 1989; Lopez-Avila, 1991). The extract was concentrated and analyzed according to EPA method 8270D for semivolatile organic compounds by selected ion monitoring (SIM) GC/MS. Detection limits for samples were elevated because of low sample volume (Appendix 1, Table S6). Insufficient volume was available to perform a matrix spike/matrix spike duplicate (MS/MSD) analysis. In lieu of this, a laboratory control sample/duplicate laboratory control sample (LCS/DLCS) analysis was performed. Internal standards of fluorene-d10, flouranthrene-d10, and p-terphenyl-d14 were used to monitor recovery and ensure that the recovery rate met acceptable standards (average of 70% or greater).

RESULTS

Patterns of Allelic Diversity.—All 16 microsatellite loci were found to be polymorphic in Louisiana, with heterozygosity

TABLE 1. Summary statistics for *Malaclemys terrapin* samples from Louisiana and comparison with a re-analysis of data from Hauswaldt (2004) and summary statistics from Hart (2005). Louisiana samples were analyzed twice: once using only the 4 loci shared with the Hauswaldt (2004) study (4L) and once using all 16 loci (16L). N = number of individuals genotyped; N_a = mean number of alleles; H_e = mean expected heterozygosity; H_o = mean observed heterozygosity; A_r = mean allelic richness; A_{priv} = number of private alleles. Private alleles were calculated using only 4 loci to enable comparison with all other terrapins (New York to South Carolina, Texas, Florida) from the Hauswaldt (2004) study.

Population	N	N_a	H_e	H_o	A_r	A_{priv}
This study						
LA (4L)	31	12.5	0.84	0.80	11.8	1
LA (16L)	31	7.38	0.62	0.56	6.98	na
Hauswaldt (2004) data, including 4 loci (all shared with this study)						
TX	14	8	0.81	0.80	9.81	0
FL	12	6.75	0.74	0.63	6.23	1
SC	171	16.25	0.88	0.86	12.5	4
NC	20	9	0.84	0.76	11.23	0
MD	56	12.5	0.80	0.79	11.25	2
NJ	26	11	0.85	0.78	9.25	0
NY	21	8	0.76	0.61	8.62	0
Hart (2005) data including 16 loci (12 shared with this study)						
TX	15	3.80	na	0.43	na	na
FL	260	4.45	na	0.39	na	na
LA	41	5.80	na	0.48	na	na
SC	50	8.30	na	0.68	na	na
NC	265	7.25	na	0.69	na	na
VA	116	6.52	na	0.67	na	na
MD	501	7.12	na	0.67	na	na
NJ	51	6.60	na	0.65	na	na
NY	31	6.50	na	0.61	na	na
MA	79	3.70	na	0.43	na	na

averaging 0.56 (Table 1). No loci were found to be in linkage disequilibrium at the $P = 0.05$ level after a Bonferroni correction for multiple tests. Departures from Hardy–Weinberg Equilibrium (HWE) were seen in two loci (D121 and D114) after Bonferroni correction. Therefore, subsequent analyses were run with and without these loci to ensure that they did not skew our results. Allelic richness for Louisiana terrapins was highest for TerpSH8 (14.23) and TerpSH1 (13.29). Heterozygosity in our Louisiana samples was high (0.8) in comparison to Hauswaldt (2004), which included samples from throughout the species range but only used four loci. However, when all 16 loci (4 shared with Hauswaldt, [2004] plus 12 shared with Hart, [2005]) were included, the heterozygosity estimate for Louisiana samples was much lower (0.56) (Table 1). When comparing sites within Louisiana using 16 loci, the average number of private alleles per individual was 1.29; however, this value may be inflated because of low sample sizes ($N < 10$ per population).

A sign test showed that, although the number of loci within Louisiana with heterozygote excess exceeds expectations, the result was not significant ($P > 0.05$). Our data best fit the assumptions of a stepwise mutation model (SMM; $P = 0.00004$). The M-ratio test showed no evidence for a population bottleneck as only two loci (D55, D62) had M ratios less than 1.0 (0.67 and 0.69, respectively). The average M-ratio for Louisiana terrapins using 16 loci was 1.15, indicative of a population at equilibrium.

Population Structure.—No population genetic structure was detected among terrapins from Louisiana using pairwise F_{st}-values between sites and 16 loci, nor when sites were grouped into "East" and "West" regions, assuming the Mississippi River as a barrier to dispersal where West $N = 11$ and East $N = 20$ (all $P > 0.05$, see Table 2). We also did not find evidence for sex-biased dispersal in Louisiana using any of the parameters tested (F_{st}, mAIc, vAIc, all $P > 0.05$). Adding Louisiana samples to previously published data did not significantly change the conclusions of Hauswaldt (2004) regarding rangewide population structure. As in that study, we found evidence for genetic structure among populations when testing alternative hypotheses using AMOVAs. However, the hypothesis that explained the largest amount of genetic variation among regions (7.47%) was the one that grouped Gulf Coast populations with East Coast populations with the exclusion of Florida (Table 3). The neighbor-joining tree best fit to pairwise chord distances revealed a hierarchical population structure, which divides Gulf Coast terrapins and Florida terrapins into two distinct regions. East Coast populations were grouped into one region because further

TABLE 2. Pairwise F_{st} comparisons (below diagonal) and associated Bonferroni-corrected P-values (above diagonal) for *Malaclemys terrapin* using data from Hauswaldt (2004) and Louisiana samples from this study at 4 shared loci. Data from outside of Louisiana are from Hauswaldt (2004).

Population	LA	SC	NC	MD	NJ	NY	FL	TX
LA		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0008
SC	0.0345		0.0005	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NC	0.0719	0.0164		0.0925	0.0002	0.0151	<0.0001	<0.0001
MD	0.0763	0.0295	0.0079		<0.0001	<0.0001	<0.0001	<0.0001
NJ	0.0746	0.0281	0.0342	0.0527		<0.0001	<0.0001	<0.0001
NY	0.1276	0.0612	0.0249	0.0570	0.0482		<0.0001	<0.0001
FL	0.1344	0.0944	0.1252	0.1430	0.1358	0.1757		<0.0001
TX	0.0354	0.0457	0.0896	0.1054	0.0918	0.1445	0.1506	

TABLE 3. Results of AMOVAs used to test alternative hypotheses about genetic structure in *Malaclemys terrapin* across its range across 4 shared loci ($N = 351$).

	All populations grouped		East Coast vs. Gulf Coast[a]		East Coast and Gulf Coast vs. Florida[b]	
	w/ TerpSH8	w/o TerpSH8	w/ TerpSH8	w/o TerpSH8	w/ TerpSH8	w/o TerpSH8
Among regions	n/a	n/a	3.91	3.09	7.47	5.34
Within regions	5.00	4.64	3.30	3.31	3.97	3.92
Within populations	95.00	95.36	92.78	93.60	88.56	90.73

[a] East Coast vs. Gulf Coast = South Carolina to New York vs. Texas + Florida + Louisiana.
[b] East Coast and Gulf Coast vs. Florida = New York to South Carolina + Texas + Louisiana vs. Florida.

structure within this region is beyond the scope of this study, and our results do not conflict with the original groupings of Hart (2005). When pairwise F_{st}-values for Louisiana were compared to Hauswaldt's (2004) rangewide dataset using 4 loci, the states with terrapins most differentiated from Louisiana were Florida (0.13438; $P < 0.0001$), and New York (0.12763, $P < 0.0001$). Louisiana terrapins were moderately differentiated from the mid-Atlantic states and were most similar to South Carolina and Texas populations (Table 2). Combining our Louisiana samples with Hauswaldt's (2004) dataset yielded similar results when testing for IBD: F_{st} was not correlated ($R^2 = 0.1788$, $P = 0.06$), and D_{SA} was weakly correlated ($R^2 = 0.24$, $P = 0.03$) with distance when Florida was included; when Florida was excluded, both F_{st} ($R^2 = 0.68$, $P = 0.001$) and D_{SA} ($R^2 = 0.76$, $P = 0.001$) showed strong and significant correlations with geographic distance. The inclusion of our Louisiana samples does not change the conclusions of Hauswaldt's (2004) study, suggesting that gene flow and dispersal patterns in Louisiana terrapins are similar to populations from other parts of the species range.

In assignment tests using the exclusion method, Louisiana was included as a possible source population for 21 of 31 terrapins from Louisiana (using a stringent probability threshold of 20%). Of these, 65% (20) were assigned to Louisiana with the highest probability (Fig. 3). The remaining 11 terrapins that were not assigned to Louisiana with the highest probability were assigned to the Carolinas (South Carolina, North Carolina) most frequently (8 terrapins), with just 2 individuals being assigned to each of the Chesapeake region (Maryland and New Jersey) and the Gulf Coast/Florida region (Fig. 3).

Blood Contaminant Analysis.—Of the 27 aromatic compounds we tested for using GC/MS, two were found to be present above the method detection limit (MDL): 2,6-dimethylnapthalene and biphenyl (Table 4). Recovery rates were determined to be within the acceptable range. On average, fluorine-d10 was recovered at a rate of 65%, fluoranthene-d10 was recovered at an average of 74%, and terphenyl-d14 at an average of 84%.

DISCUSSION

Genetic Structure within Louisiana.—In agreement with previous studies on genetic connectivity within large estuarine systems, we found weak to no evidence for genetic structure within Louisiana marshes, even among sites that were up to 120 km apart (Hauswaldt and Glenn, 2005; Sheridan et al., 2010). Despite 5 months' trapping attempts and by-catch collection at more than 10 localities across the Louisiana coast, we were only able to capture and sample terrapins from 4 localities within Louisiana. Although our capture rate per sampling effort was far smaller than terrapin studies from other regions (W. Roosenburg, pers. comm.), sampling density is on par with previous studies in this species where only a few sites per region were included (Hausewalt, 2004; Hart, 2005). Given that terrapins travel large distances during their lifetimes and that this and previous studies found no evidence for fine-scale genetic structure in this species, it is unlikely that the addition of samples from other Louisiana localities would affect the main conclusions of this study. Even when we assumed that the Mississippi River was a significant barrier to dispersal and grouped all western Louisiana samples (Cocodrie and Port Fourchon, $N = 11$) against all eastern samples

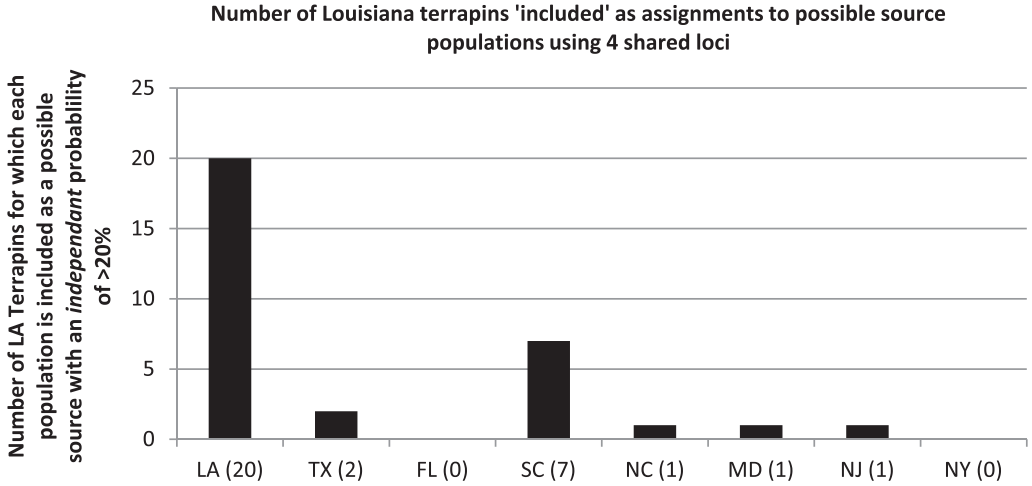

FIG. 3. Independent probability of assignments of Louisiana terrapins using 4 loci. Numbers in parentheses indicate the number of terrapins captured in Louisiana waters that were assigned with the highest independent probability to a population (by state). Genetic data for states other than Louisiana are from Hauswaldt (2004).

TABLE 4. Aromatic chemicals detected in pooled *Malaclemys terrapin* plasma samples from 3 Louisiana locations.

Compound detected (ug/kg wet weight)	Population		
	Cx	LMx	SBx
2,6-Dimethylnapthalene	0.74	0.34	0.23
Biphenyl	0.72	0.67	0.61
Total	1.46	1.01	0.84

(Lake Machias and Shell Beach, $N = 20$), F_{st} and other measures of genetic differentiation were not significant.

Although long-term mark–recapture studies have shown terrapins in some populations to have high site fidelity to tidal creeks and rivers (Gibbons et al., 2001; Roosenburg et al., 1999), this study, as well as previous studies of genetic structure within South Carolina and New Jersey estuaries, indicate that site fidelity is unlikely to be the major force driving genetic structure and gene flow (Hauswaldt and Glenn, 2005; Sheridan et al., 2010). Our results support previous findings that larger-scale migration by both males and females to mating aggregations, as well as female movement (up to 8 km; Hurd et al., 1979) to nesting beaches, may be a significant contributor to gene flow across large landscapes (Hurd et al., 1979; Hauswaldt and Glen, 2005; Sheridan et al., 2010). Terrapins gather into large mating aggregations in March and April, and are easily observed by boat (pers. comm. with W. Roosenburg and Louisiana fishing communities, see also Hauswaldt and Glenn, 2005). Although terrapins may have high site fidelity through the off-breeding season, they likely travel great distances to mating aggregations in the spring, potentially accounting for their lack of genetic structure over great geographic distances (Roosenburg et al., 1999; Gibbons et al., 2001; Hart, 2005).

Mark–recapture studies suggest that the maximum distance that females travel is 8–10 km (Hurd et al., 1979; Gibbons et al., 2001). However, it is possible that the loss of suitable nesting habitat resulting from coastal erosion, oil spills, and subsidence along the Gulf Coast forces females to travel longer distances to find suitable nesting sites. Predation on juvenile terrapins and short-term dispersal has been recorded in the high marsh areas, mainly behind nesting beaches. However, little is known about juvenile dispersal and how the movement of this life-history stage affects gene flow (Draud et al., 2004). In addition, longer-term processes such as recolonization after glacial maxima may contribute significantly to genetic patterns (Walker and Avise, 1998; Wares, 2002).

Although past research has detected male-biased dispersal in terrapins (Hart, 2005; Sheridan et al., 2010), we found no evidence for sex-biased dispersal in Louisiana ($N = 31$) using 16 microsatellite loci. It is worth mentioning that, unlike populations in other localities where sampling has been consistently female skewed, individuals encountered in Louisiana were predominantly male (20 males: 9 females: 2 museum tissue unknown). This pattern is consistent with results from sampling in Louisiana in the 1950s (Cagle, 1952; Roosenburg, 1991; Hart, 2005; Hauswaldt, 2004). Previous studies have suggested that the combination of a sex-ratio bias and male-biased dispersal may drive genetic mixing, despite female nesting fidelity (Sheridan et al., 2010). Also, our male-biased sex ratio could have resulted from a difference in sampling procedure. Although previous studies have used hoop nets, fyke nets, dip nets, and hand capture in the spring to early summer (Hauswalt, 2004; Sheridan et al., 2010), we sampled in the late summer, fall, and early winter and were primarily successful using trawling nets on large shrimp boats in deep waterways. Applying this technique to previously studied sites may help to better understand the relationship between sampling technique and observed sex ratios. Further investigation is needed to determine whether the lack of evidence of male-biased dispersal and large proportion of males captured in this study is the result of a distinct natural history for Gulf Coast terrapins.

Relationship of Louisiana Terrapins to Other Regions.—As shown in previous studies, a small degree of hierarchical genetic structure can be seen across the Diamondback Terrapin's range (Lamb and Avise, 1992; Hauswaldt, 2004; Hart, 2005). When considering pairwise F_{st}-values obtained using 4 loci shared between our study and Hauswaldt's (2004), Louisiana terrapins appear to be more closely related to terrapins from the Carolinas than to Florida. According to criteria suggested by Wright (1978) (F_{st}: 0.00–0.05 = little, 0.05–0.15 = moderate, 0.15–0.25 = great), Louisiana, South Carolina, and Texas populations show little genetic differentiation. Louisiana populations show moderate genetic differentiation when compared to all other states (Maryland, New Jersey, North Carolina, New York, and Florida), with Florida and New York being the most differentiated.

Using AMOVAs to test multiple alternative hypotheses for regional genetic structure across 4 loci, we found that the largest amount of genetic variation among regions (7.47%) was explained when Florida was grouped separately from all other populations (Table 3). This corroborates Hauswaldt's (2004) findings that Texas (Gulf Coast) terrapins are more closely related to East Coast terrapins than they are to terrapins from Florida and lends further support to our hypothesis that Louisiana terrapins are genetically similar to East Coast terrapins, which were the likely source of translocations during the early 1900s. Furthermore, the neighbor-joining tree, best fit to pairwise chord distances D_{CE}, showed a hierarchical regional structure in which Gulf Coast terrapins from Texas and Louisiana form one grouping separate from Florida (Fig. 2). East Coast terrapins were grouped into one region, because further resolution of this region is beyond the scope of this study. When comparing genetic and geographic distances, all parameters except one (D_{SA}) showed a significant pattern of isolation by distance (IBD) only when Florida was excluded, and all correlation coefficients increased when Florida samples were excluded. Although our sampling does not permit us to comment on the delineation of management units (MUs) outside of the Gulf region, it does support the grouping of Louisiana and Texas terrapins into one MU, a recommendation first proposed and supported by data from Hart (2005), and the lumping of these states' recognized subspecies (*M. t. pileata* in Louisiana and *M. t. littoralis* in Texas) into one. Because our study did not include any terrapins from northwest Florida, we are unable to determine whether these should also be grouped into a "Gulf Coast" MU or whether they are as genetically distinct as the individuals from the Florida Keys.

Although our rangewide analyses were performed on only 4 loci and some of our sites had small sample sizes, our results were consistent with those of Hauswaldt (2004), namely that Gulf Coast terrapins are genetically more similar to East Coast populations than to their closer neighbors in the Florida Keys. As has been mentioned, this is likely attributable to the large displacement and translocation of turtles in the early 20th century when these turtles were hunted and farmed (Coker, 1920). Overharvesting of this species across its range led to a dramatic decline around the turn of the 20th century (Hilde-

brand and Hatsel, 1926). When East Coast populations of terrapins became too scarce, wild stocks as well as farms were replenished with turtles from the Carolinas and the Gulf Coast (Coker, 1920). Many farms were also established in the Gulf Coast in response to the native population being overharvested. This includes the farm purported to be the largest, in the marshes north of Dauphin Island, Alabama (T. Wibbels, unpubl. data). Some of these farms still exist in areas of southern Louisiana, although on a much smaller scale (pers. comm. to authors from local Louisiana fishing communities). The source populations of terrapins for these farms is not known; however, it is likely that they were brought in from places where terrapins were captured in abundance (e.g., the Carolinas) after overharvesting had depleted local populations. Because many of these farms were small and privately owned, there are no records as to the number or origin of terrapins relocated during this period of time (Coker, 1920).

Our assignment tests may be a reflection of this mixing, because 11 of 31 Louisiana terrapins were assigned to East Coast rather than Gulf Coast source populations with the highest probability (Fig. 3). These results suggest that when considered in the context of genetic variation across the species range, Louisiana terrapins share more genetic similarities with East Coast terrapins than with their closer neighbors in Texas and Florida. Although this conclusion is based on few loci, assignment tests suggest genotypes from the East Coast were found more frequently in Louisiana than were genotypes from either Texas or Florida. We are unaware of any large terrapin farms that existed in Texas; however, the genetic diversity found there may be a result of gene flow throughout the Gulf Coast since the translocations occurred. Genetic analysis of terrapins from western Louisiana (Cameron Parish) would be informative in delineating gene flow and differentiation between these two populations. Additional sampling along the Florida coast would also help to clarify the relationship between East and Gulf Coast populations and may shed light on possible ecological barriers to gene flow.

Conservation Implications.—Like other Gulf Coast terrapin populations, those found in Louisiana are thought to be declining (Seigel and Gibbons, 1995). Using 16 microsatellite loci, we found no evidence of a recent population bottleneck using Wilcoxon sign-rank testing or using an M-ratio test to quantify lost alleles. However, our data were best fit to the SMM, which has been known to mask heterozygosity excess even when a bottleneck is known to have occurred (Cornuet and Luikart, 1996). Adding Louisiana samples to data from Hauswaldt (2004) at 4 shared loci, we found that genetic diversity is higher in southern populations (with the exclusion of Florida) than in northern ones. This pattern has been documented for many taxa whose range extends from the northern Atlantic coast along the eastern seaboard and down across the Florida Peninsula to the Gulf Coast, and is hypothesized to be the result of recolonization of northern areas after the last glacial maximum from genetically diverse southern refugia (Walker and Avis, 1998; Wares, 2002). However, when considering genetic variation within Louisiana across all 16 loci, we found low genetic diversity in Louisiana in comparison to values from previous studies of terrapins across their range (Hart, 2005; 16 loci, 12 shared with our analysis, see Table 1). Although this genetic diversity estimate for Louisiana could have been affected by our small sample size ($N = 31$ terrapins), we consider this unlikely given that several studies examining the effects of small sample sizes for microsatellite data demonstrate that 20–30 individuals are sufficient to estimate diversity indices accurately (Pruett and Winker, 2008; Hale et al., 2012). Because our finding of low genetic diversity in Louisiana deviates from the pattern observed in other similarly distributed taxa (Walker and Avis, 1998; Wares, 2002), it suggests that a long-term decline in genetic diversity, perhaps as a result of regional population declines, may be occurring. Tests used here such as the Wilcoxon sign-rank or M-ratio use ratios of allele range to number of alleles and relative measures of heterozygote excess to detect recent bottleneck events. Population declines over a long period of time could have caused a reduction in both the range of alleles and number of alleles, as well as evened out the ratio of heterozygotes. Because available methods are designed to detect shorter-term bottleneck events, it is possible that a longer-term decline might have occurred but would not be detected by our bottleneck analysis.

Conversations we had with Louisiana fishing community members, who aided in identifying and sampling terrapin populations during this study, support the hypothesis of a long-term decline of terrapins in Louisiana. Our contacts in Shell Beach, Lake St. Catherine, Cocodrie, and Port Fourchon all reported that terrapins were once abundant but now appear to have a more restricted range and are less frequently encountered as by-catch. Further studies of Louisiana terrapin populations are needed to corroborate or refute this anecdotal evidence. If in fact there has been a decline, a number of factors, including intense hunting in the early 20th century, habitat loss, and contamination of nesting beaches by anthropogenic pollutants may have been important contributors.

Blood Contaminant Loads after the Deepwater Horizon Oil Spill.—We found total contaminant levels to be greater at sites that were less protected by barriers, received more oiling, and had more reports of stranded marine animals (Cocodrie) than in sites with moderate (Lake Machias) or low (Shell Beach) incidences of oiling and marine animal strandings. However, our sample size ($N = 3$ sites) was too small to merit statistical testing for this comparison (Appendix 1, Figs. S4 and S5 online). Unfortunately, we were unable to recover usable plasma or liver samples from Port Fourchon, our most heavily oiled site, because the turtles from that site were found dead and were already too decomposed. Although testing for PAHs and other aromatic hydrocarbons is more accurately done by sampling fat and liver (because these compounds are lipophilic, and liver samples often provide recovery of PAH metabolites), PAHs have been successfully recovered in plasma samples from oiled birds and sea turtles, and plasma collection is far less invasive than liver sampling (Troisi and Borjesson, 2005; Camacho et al., 2012). In previous studies, plasma PAH concentrations have been found to be as high as 2,000 μg/kg in heavily oiled birds (Troisi and Borjesson, 2005). However, these measurements included PAH metabolites, from which limited funding precluded us. Of the 27 chemicals included in our study, 18 overlapped with panels used in previous studies to test for PAHs in terrapin eggs and Loggerhead Sea Turtle plasma; however, none of the aromatic compounds identified in our study were the same as those found previously in terrapin eggs or Loggerhead Sea Turtle plasma (Holliday et al., 2008; Camacho et al., 2012).

PAHs and other aromatic compounds are known to exist naturally in soil as a result of the burning and decomposition of organic matter (Neff et al., 2005). However, the 2-ring aromatic hydrocarbon biphenyl and the alkylated PAH dimethylnapthalene found in this study are most commonly associated with crude oil and gasoline (Neff et al., 2005). Furthermore, tests that use panels of PAHs likely underestimate total concentrations by

excluding most alkylated PAHs and PAH metabolites, especially when testing within biological matrices such as plasma (Troisi and Borjesson, 2005; Neff et al., 2005). Because most of our samples were taken from turtles in deep water it was not possible to take paired soil samples to forensically match PAHs to a specific source. Consequently, although our results suggest a correlation between terrapin plasma contaminant levels and levels of oiling across sites, it is not possible to determine whether these contaminants resulted from the recent *Deepwater Horizon* oil spill, previous spills, background leakage, the petroleum distilling industry, or recreation.

Biphenyl is an aromatic hydrocarbon found in crude oil, coal tar, and natural gas and is associated with wood preservation and production plants as well as municipal waste disposal sites, although it has only been detected in close proximity to waste dumps and industrial centers (WHO/IPCS, 1999). Because biphenyl is not known to be persistent or bioaccumulate, and has been shown to have a relatively short depuration period, it is likely that the source of the biphenyl detected in this study is from a relatively recent and significant depositional event (WHO/IPCS, 1999). Additionally, because none of our sites are in direct vicinity of a municipal waste site or industrial plant (the closest is 15 km from Shell Beach), and biphenyl was present at all sites, it is more likely that a largely distributed pollution source such as an oil spill or natural seepage is responsible for the biphenyl detected in this study.

Methyled PAHs such as dimethylnapthalene are more hydrophobic, making them prone to longer retention in fatty tissues and are estimated to have marginally longer half-life times than their parent PAHs (Rantamaki, 1997). A hydrocarbon exposure study using the bivalve *Mytilus edulis* supports the idea that most PAHs are metabolized and excreted rapidly, although a small number persist in tissues and are depurated slowly (Rantamaki, 1997). For the same bivalve species, studies have shown that elimination time can vary from 4 days to several weeks, with the major determining factor being exposure period (Farrington et al., 1982; Rantamaki, 1997). Chronic exposure to hydrocarbons causes this discharge process to be slower, allowing bivalves and, thus, predators like terrapins to accumulate hydrocarbons into more stable body compartments such as membranes and circulating lipids (Rantamaki, 1997). In general, the retention times and depuration rates of PAHs in bivalves from areas that have experienced oil spills is long (a year or more) and are consistent with chronic exposure (Rantamaki, 1997). In a study examining PAHs in Gulf Coast seafood after the *Deepwater Horizon* oil spill, a decline in PAHs was found after August 2010 for oysters and crabs, but levels remained constant for fish and shrimp until January 2011 (Xia et al., 2012). Because samples tested for PAHs in this study were collected at different times, with Cocodrie samples taken mostly in late September through October, and Lake Michias and Shell Beach samples taken through November 2010 (Appendix 1, Table S5) it is possible that differences in detected contaminants could be attributable to the metabolism of these compounds by terrapins and their prey.

Although the amount of alkylated napthathlenes in Macondo 252 crude oil from the *Deepwater Horizon* oil spill is relatively high compared to other alkylated PAHs, this was not necessarily the most prevalent alkylated PAH in weathered sediment or marsh mouse samples tested (Liu et al., 2012). Additionally, biphenyl was not found in high concentration in submerged oil mats tested in three gulf coast locations after the *Deepwater Horizon* oil spill (OSAT, 2010). It is important to note, however, that the PAHs and hydrocarbons in sediment may not represent those that are most biologically available and present in free plasma lipids in higher vertebrates. In a study examining PAH levels in Gulf Coast seafood after the *Deepwater Horizon* oil spill, napthalene was found in the highest concentration in fish, shrimp, and oysters; 2-methylnapthalene, phenanthrene, and biphenyl were found above the detection limit in 80% of fish, shrimp, crab, and oysters tested; and dimethylnapthalene was found in a several seafood species (Xia et al., 2012). Thus, although it is possible that the PAHs detected in this study were a result of the *Deepwater Horizon* oil spill, we do not have sufficient evidence to state this conclusively.

Total contaminant levels detected in this study were low, even compared to background levels identified in some studies (Troisi and Borjesson, 2005; Holliday et al., 2008). Additionally, because of our small sample volume, the minimum detection limit was elevated, which may have caused us to underestimate actual contaminant concentrations. Because it is only possible to safely extract a small amount of plasma from these turtles, and because the compounds of interest are highly lipophilic, plasma may be a suboptimal indicator of contaminant load in small reptiles. Given that Holliday et al. (2008) found higher levels of PAHs in terrapin eggs after a minor oil spill, it is possible that other tissues would have harbored higher PAH levels. It is also possible that our samples were taken too soon after the spill for contaminants to have been sequestered in higher predators such as terrapins at detectible concentrations. A delayed or seasonal effect also may be present, because these samples were taken in the fall to early winter. The process of bioaccumulation of these compounds in liver or fatty tissue may be reflected in plasma more or less based on seasonal nutritional and hormonal cycles (Monteverdi and Guilio, 2000). Additionally, complete vitellogenesis can take more than a year (Kuchling, 1999). As a result, the development of generational effects is likely to be delayed one or more years, considering the time it takes for contaminants to become established in prey foods and later sequestered into fatty tissues to be passed on to offspring. It will be important to survey populations in oiled areas in future breeding seasons to ascertain the true long-term effects of the *Deepwater Horizon* oil spill on breeding, population genetics, and resilience.

Future Research and Practical Applications.—*Malaclemys terrapin* is listed as a species of special concern in Alabama, Mississippi, and Louisiana, although an open season still exists for the species in both Mississippi and Louisiana (Mirarchi et al., 2004; Watters, 2004). Conversely, terrapins are not listed at all in Texas, and hunting is only regulated as a nongame species, with no specified open season. Previous work has shown that in addition to exhibiting genetic structure at the population level (as shown with significant pairwise F_{st}-values), terrapins also exist in genetically distinct metapopulations (Fig. 2), which represent statistically significant changes in allele frequencies (see measures of IBD and genetic distance from this study and AMOVA analysis from Hauswaldt [2004] and Hart [2005]). These regional groupings are supported by several measures of genetic distance in this study, and have been supported previously by Hart (2005) and Hauswaldt (2004). Our study echoes the recommendations of Hart (2005) that Gulf Coast terrapins (encompassing the subspecies *M. t. pileata* in Louisiana and *M. t. littoralis* in Texas, which were defined using carapace morphology and skin coloration by Ernst et al., 1994 and Carr, 1952) be lumped together as one MU. Although some subspecies boundaries have fit moderately well with genetic frequencies, Gulf subspecies

boundaries for *M. t. pileata*, *M.t. macrospilota*, and *M.t. littoralis*, originally based on morphology that has since been shown to be variable even within sites (Hart, 2005), have not been corroborated with genetic evidence (Hart, 2005). Additionally, because reduced genetic diversity is apparent at the regional scale, rather than at the population scale, it indicates that 1) intraregional dispersal may be important, and 2) the region is more likely the relevant level at which to manage populations. We find genetically distinct regional groupings to be more biologically relevant to policy makers than the existing subspecies delineations. Despite the potential for population mixing associated with terrapin aquaculture, and the genetic signal still present from this event, Texas and Louisiana terrapins are still supported strongly as a regional grouping in this and previous work (Fig. 2; Hart, 2005). Because we are not aware of any aquaculture that has moved large numbers of terrapins between Louisiana and Texas, and because there are regional genetic groupings in other areas in their range, it is prudent to assume that this grouping is most likely a result of genetic mixing via natural dispersal between Louisiana and Texas. Other studies have shown that male-biased dispersal may be a significant driver of interpopulation genetic mixing and may be the reason that terrapins lack the more distinct genetic structure that typifies other philopatric species (Hart, 2005; Sheridan et al., 2010). Additional sampling in other Gulf Coast states, including the northwest peninsula and western panhandle of Florida, will clarify whether the Gulf Coast MU should include western Florida or whether these terrapins are as distinct as Florida Keys individuals. Moreover, extensive sampling of regions bordering Texas and Louisiana will further clarify the degree of change along this genetic cline and potentially help define ecological boundaries between Gulf Coast and Florida populations. Despite historic translocations, we were able to detect a population-genetic signal of IBD as well as population differentiation; therefore, we believe the proposed MUs will provide the most biologically relevant guidelines for conservation and management.

Our results also suggest that Gulf Coast terrapins may not have recovered from severe overharvesting events in the early 20th century because they display reduced genetic diversity when compared to populations in other parts of the species range. In addition to other anthropogenic threats such as crab pots, introduced species (nest predation by coyotes, *Canis latrans*), loss of habitat, fragmentation, road mortality, and overharvesting, terrapins may also be at increased risk because of exposure to chemical contaminants after events such as the *Deepwater Horizon* oil spill. Given the results of this study and the multitude of threats faced by this species, we recommend that *M. terrapin* within the Gulf Coast region (especially in Louisiana) be considered as at least a species of special concern and that no open season exists for this species within this region. Because of our small sample size, research to add support and possibly extend these recommendations within Louisiana is needed and will help to clarify this species' status. However, given the extreme difficulty we experienced in locating and capturing this species, the data presented here, the multitude of known threats to terrapins in Louisiana, and anecdotal evidence of population declines, we have chosen to err on the side of caution in assessing the potential threatened status of this species. Monitoring of chronic, genetic, and reproductive effects of contaminant exposure also will be important in assessing the health of this species in the future, as well as estimating the expected impacts of other potential threats to terrapins and their habitats. Implicit in these types of assessments is the need for more baseline genetic and chemical data for terrapins, especially across the Gulf Coast.

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