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GEOGRAPHICAL VARIATION OF PERSISTENT ORGANIC POLLUTANTS IN EGGS OF THREATENED LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) FROM SOUTHEASTERN UNITED STATES

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Abstract—Persistent organic pollutants (POPs) are recognized manmade threats to sea turtle populations, but substantial uncertainty exists surrounding their exposure to contaminants and their sensitivity to toxic effects. This uncertainty creates difficulty for conservation managers to make informed decisions for the recovery of these threatened species. To provide baseline concentrations and spatial comparisons, we measured a large suite of POPs in loggerhead sea turtle (*Caretta caretta*) egg yolk samples collected from 44 nests in three distinct U.S. locations: North Carolina (NC), eastern Florida (E FL), and western Florida (W FL). The POPs included polychlorinated biphenyls (PCBs), organochlorine pesticides such as dichlorodiphenyltrichloroethanes (DDTs), chlordanes, mirex, dieldin, hexachlorocyclohexanes (HCHs), hexachlorobenzene, and toxaphene congeners, as well as polybrominated diphenyl ether congeners (PBDEs). Persistent organic pollutant concentrations were lowest in W FL, intermediate in E FL, and highest in NC egg samples, with several statistically significant spatial differences. This increasing gradient along the southeast coast around the Florida peninsula to North Carolina was explained partly by the foraging site selection of the nesting females. Data from previous tracking studies show that NC nesting females feed primarily along the U.S. eastern coast, whereas W FL nesting females forage in the Gulf of Mexico and Caribbean Sea. The E FL nesting females forage in areas that overlap these two. The foraging site selection also results in exposure to different patterns of POPs. An unusual PBDE pattern was seen in the NC samples, with nearly equal contributions of PBDE congeners 47, 100, and 154. These findings are important to managers assessing threats among different stocks or subpopulations of this threatened species. Environ. Toxicol. Chem. 2011;30:1677–1688. © 2011 SETAC

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INTRODUCTION

Environmental contaminants are a recognized threat to many species, yet the uncertainty of the risk they pose to sea turtles is great, and our understanding is limited by few data [1]. Here we focus on contaminants in loggerhead sea turtle (*Caretta caretta*) eggs. Loggerhead sea turtles are currently listed as threatened on the U.S. Endangered Species List. However, the Northwest Atlantic subpopulation has been experiencing a long-term declining trend in nesting, which has heightened concern for the species and compelled National Marine Fisheries Service to consider this subpopulation for the more imperiled status of endangered ([2]; http://www.nmfs. noaa.gov/pr/pdfs/statusreviews/loggerheadturtle2009.pdf). Four recovery units (aggregations of loggerhead sea turtles, essential to the recovery of the species and delineated based partly on geographical isolation) have been identified for rookeries for the Northwest Atlantic loggerhead in the United States: the Northern nesting subpopulation (ranging from Virginia to northern Florida), the Peninsular Florida subpopulation, the Northern Gulf of Mexico subpopulation, and the Dry Tortugas subpopulation [1]. The first two subpopulations were sampled in the present study. The Northern subpopulation has been declining by ≈1.6% per year since the 1980 s [1]; the Peninsular Florida subpopulation has experienced a cumulative decline of 28 to 31% between 1989 to 2006 [3]. The list of threats that this species faces is long, including nesting beach habitat destruction, fisheries by-catch, vessel strikes, poaching, diseases, predation, marine debris, and chemical pollutants.

Baseline exposure data do not exist for contaminant concentrations in certain sea turtle subpopulations. For example, the eastern coast of Florida hosts possibly the largest rookery of loggerheads in the world, rivaled only by Masirah in Oman [1], but only three loggerhead nests from this location have been analyzed recently for persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides [4]. Before that study, loggerhead eggs from this region had not been collected for POP measurements since the 1970 s [5], and the analytical methods used three decades ago are now considered obsolete. Since the 1990 s, loggerhead eggs from the United States have been analyzed for POPs from only South Carolina [6], the Florida panhandle [7], and eastern Florida [4]. These three studies are similar in that they used nonlethal sampling of unhatched eggs collected after live hatchlings emerged from the nest. Despite this similarity, the data cannot be combined for a robust spatial comparison of POP exposure among the genetically distinct subpopulations or regions because of temporal differences in sampling (1970 s to 2002), differences in the suite of compounds measured, and methodological differences (e.g., Alam and Brim [7] reported on dry mass, whereas all other studies used wet mass).

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During a spring–summer nesting season, loggerhead turtles lay three to six clutches 14 d apart of 100+ eggs each on nesting beaches [8,9]. Every two to three years, females migrate to the nesting beaches hundreds of kilometers from foraging grounds, and often they choose the same foraging ground between nesting seasons [8]. During this foraging time, the nutritional resources of the local environment are deposited into follicles (which become the yolk of the egg) for the next nesting season [9]. There, adult females accumulate POPs from their prey, as well as from incidentally ingested sediments, that then are deposited, along with lipids, into follicles. Indeed, maternal transfer of POPs into eggs has been documented in many turtle species, including sea turtles [10–13]. Thus, the POP concentrations in eggs represent contamination received on foraging grounds of the adult female. Females nesting on the same beach but foraging in different locations would likely produce eggs containing different POP concentrations. Alternatively, if females from one nesting beach forage in similar locations, then their egg POP concentrations would be similar and indicative of their foraging region. Therefore, knowing the exposure level of a species to these compounds as well as the spatial structure at the subpopulation or regional level is important to make informed management decisions for their population recovery.

Several studies have indicated that the presence of POPs in eggs represents a risk to the developing embryo. In green sea turtles (*Chelonia mydas*), van de Merwe et al. [12] showed that POPs transfer from eggs into embryos and that higher egg POP concentrations correlated with a lower mass: length ratio of the hatchlings. Although more studies are needed to prove that POPs are indeed the cause of this observation, turtles with a lower body condition index may not be as fit to survive early migrations and avoid predators. Additionally, toxic effects of POPs on the very sensitive early life stages have been shown in a number of other reptile species (e.g., [14–20]).

Objectives of the present study were to provide baseline concentrations of a large suite of POPs, including PCBs, dichlorodiphenyltrichloroethane (DDT)-related compounds, chlordanes, toxaphenes, mirex, dieldrin, hexachlorocyclohexanes (HCHs), and hexachlorobenzene, as well as brominated flame retardants, the polybrominated diphenyl ethers (PBDEs) in loggerhead eggs collected in distinct nesting regions along the U.S. southeast coast: western Florida (W FL), eastern Florida (E FL), and North Carolina (NC). Spatial differences in POP concentrations and patterns were interpreted based on previously published reports of nesting female migrations from nesting locations similar to their preferred foraging grounds.

MATERIALS AND METHODS

Egg collection and selection

Egg sampling was conducted in collaboration with a large-scale project to evaluate sex ratios on nesting beaches in the southeastern United States in 2002 [21]. Individual nests were located most often the morning after laying without encountering the female. Nests were marked with signs or predator-exclusion cages and observed over the incubation period. These methods ensure that multiple nests do not mix, but they do not rule out the chance, however small, that successive clutches from one female may have been sampled. Eggs that failed to hatch were collected into plastic bags during nest inventories. Although the decision to use unhatched eggs may introduce some variability because of different stages of embryonic development and different degrees of degradation, this choice provides a nonlethal sampling method and has proved useful in many other sea turtle studies (see especially Stewart et al. [13]). Eggs were collected from a total of 44 nests at three regional locations (Table 1; Fig. 1), making this study the second largest sample size of sea turtle nests analyzed for POPs, second to Guirlet et al. [10]. Nests from Sarasota County, FL (FLSA; $n = 11$) were considered from W FL. Nests from Boca Raton (FLBR; $n = 11$), Juno Beach (FLJU; $n = 4$), Hutchinson Island (FLHI; $n = 5$), and Melbourne Beach (FLME; $n = 4$) were grouped as from E FL. Nests from Cape Lookout, NC (NCCL; $n = 9$) were considered from NC. Eggs were rinsed inside a fume hood with deionized water to remove sand, opened, and staged to determine the extent of embryonic development. Because egg contents were split for sex determination and contaminant measurements, we decided to store and analyze only yolk for contaminants. Yolk was separated from the albumen as much as possible and stored frozen in hexane-rinsed aluminum foil. One to ten yolk samples from eggs with no development or early to middle developmental stages were pooled per nest (50% or less of eggs from single nests were from middle stage; Table 1). Samples from late-stage development embryos were excluded to minimize confounding factors, because POP concentrations are known to become more concentrated in loggerhead yolk samples with embryonic development, especially by the late stage [4]. Two nests with only one egg each were included, because good agreement in POP concentrations has been shown among loggerhead egg yolk samples from a single nest (at least among eggs with no, early, or middle stage development, where average relative standard deviation for total POPs was 14% [4]). This low variability within a nest has been shown in eggs from other sea turtle species [12]. Three nests, included in the 44 nests mentioned, were previously analyzed as individual yolk samples rather than pooled ([4]; see Table 1), and the average POP concentrations of those no, early, and mid-developmental stages were included in the present study.

Calibration solutions and quality control

Calibration solutions were prepared gravimetrically in iso-octane by combining National Institute of Standards and Technology Standard Reference Materials (SRMs): 2261 Chlorinated Pesticides in Hexane, 2262 Chlorinated Biphenyl Congeners in 2,2,4-Trimethylpentane, 2274 PCB Congeners Solution II in Isooctane, 2275 Chlorinated Pesticides Solution II in Isooctane, as well as solutions containing 46 additional PCB congeners and 14 PBDE congeners (PBDE solution from Cambridge Isotope Laboratories). A six-point calibration curve, ranging from 0.35 ng to 370 ng of each compound contained in the previously mentioned solutions, was extracted and processed alongside the samples. A three-point calibration curve consisting of four toxaphene compounds (0.03–0.5 ng) was also prepared gravimetrically but not extracted alongside samples, to semiquantitatively determine concentrations of the following: 2-*endo*, 3-*exo*, 5-*endo*, 6-*exo*, 8, 8, 10, 10-octachlorobornane (Parlar 26), 2-*endo*, 3-*exo*, 5-*endo*, 6-*exo*, 8, 8, 9, 10, 10-nonachlorobornane (Parlar 50), 2, 2, 5, 5, 8, 9, 9, 10, 10-nonachlorobornane (Parlar 60), and 2-*endo*, 3-*exo*, 6-*exo*, 8, 9, 10, 10-heptachlorobornane (Parlar 32). An internal standard solution in iso-octane was added (~40 ng of each compound) gravimetrically to samples and the calibration standards before extraction and contained 4,4′-DDT-d_8, 4,4′-dichlorodiphenyldichloroethylene-d_8 (4,4′-DDE-d_8), 4,4′-dichlorodiphenyldichloroethane-d_8 (4,4′-DDD-d_8), endosulfan I-d_4, PCB 103, and PCB 198. National Institute of Standards and Technology SRM 1946 Lake Superior Fish

Table 1. Loggerhead sea turtle nest locations (all in USA) and sample size information

Nest	Island/Beach	Region	Recovery Unit	Date Collected	Number of yolks pooled	% from mid-development
FLSA02	Sarasota area	WFL	Peninsular FL	July 26, 2002	4	0
FLSA04	Sarasota area	WFL	Peninsular FL	July 26, 2002	8	0
FLSA05	Sarasota area	WFL	Peninsular FL	July 30, 2002	2	0
FLSA06	Sarasota area	WFL	Peninsular FL	August 14, 2002	3	0
FLSA08	Sarasota area	WFL	Peninsular FL	August 23, 2002	4	0
FLSA09	Sarasota area	WFL	Peninsular FL	August 13, 2002	5	0
FLSA10	Sarasota area	WFL	Peninsular FL	August 14, 2002	3	0
FLSA11	Sarasota area	WFL	Peninsular FL	September 4, 2002	3	0
FLSA12	Sarasota area	WFL	Peninsular FL	Sept. 12 & 16, 2002	4*	25
FLSA14	Sarasota area	WFL	Peninsular FL	Sept. 12 & 16, 2002	2	0
FLSA15	Sarasota area	WFL	Peninsular FL	Sept. 12 & 24, 2002	3	0
FLBR02	Boca Raton	EFL	Peninsular FL	July 19, 2002	6*	50
FLBR05	Boca Raton	EFL	Peninsular FL	July 19, 2002	6	0
FLBR07	Boca Raton	EFL	Peninsular FL	August 9, 2002	4	0
FLBR08	Boca Raton	EFL	Peninsular FL	August 8, 2002	3	0
FLBR09	Boca Raton	EFL	Peninsular FL	August 8, 2002	1	0
FLBR10	Boca Raton	EFL	Peninsular FL	August 11, 2002	5	0
FLBR11	Boca Raton	EFL	Peninsular FL	September 7, 2002	3	0
FLBR12	Boca Raton	EFL	Peninsular FL	September 7, 2002	2	0
FLBR13	Boca Raton	EFL	Peninsular FL	September 12, 2002	10	0
FLBR14a	Boca Raton	EFL	Peninsular FL	September 21, 2002	4*	50
FLBR15	Boca Raton	EFL	Peninsular FL	September 17, 2002	2	0
FLHI04	Hutchinson Island	EFL	Peninsular FL	July 26, 2002	3	0
FLHI09	Hutchinson Island	EFL	Peninsular FL	August 13, 2002	3	0
FLHI10	Hutchinson Island	EFL	Peninsular FL	August 27, 2002	5	20
FLHI11	Hutchinson Island	EFL	Peninsular FL	September 17, 2002	3	0
FLHI14	Hutchinson Island	EFL	Peninsular FL	September 23, 2002	3	0
FLJU06	Juno Beach	EFL	Peninsular FL	August 12, 2002	6	0
FLJU10	Juno Beach	EFL	Peninsular FL	August 12, 2002	4	0
FLJU12	Juno Beach	EFL	Peninsular FL	September 22, 2002	1	0
FLJU13	Juno Beach	EFL	Peninsular FL	September 22, 2002	3	0
FLME07	Melbourne Beach	EFL	Peninsular FL	August 14, 2002	4	0
FLME09	Melbourne Beach	EFL	Peninsular FL	August 14, 2002	6	33
FLME10	Melbourne Beach	EFL	Peninsular FL	August 12, 2002	6	40
FLME14	Melbourne Beach	EFL	Peninsular FL	September 19, 2002	3	0
NCCL01	Cape Lookout	NC	Northern	August 16, 2002	6	17
NCCL04	Cape Lookout	NC	Northern	August 16, 2002	2	0
NCCL05	Cape Lookout	NC	Northern	August 19, 2002	3	0
NCCL11	Cape Lookout	NC	Northern	August 19, 2002	3	0
NCCL12	Cape Lookout	NC	Northern	August 23, 2002	2	0
NCCL13	Cape Lookout	NC	Northern	August 23, 2002	7	0
NCCL14	Cape Lookout	NC	Northern	August 30, 2002	4	0
NCCL15	Cape Lookout	NC	Northern	August 30, 2002	6	33
NCCL21	Cape Lookout	NC	Northern	October 4, 2002	3	0

* Number of individual yolk samples averaged from Alava et al. [4].
W FL = western Florida, USA; E FL = eastern FL, USA; NC = North Carolina, USA.

Tissue (hereafter SRM 1946) and a cryohomogenized composite of loggerhead sea turtle egg yolks from nest FLBR13 were analyzed as control materials. Three procedural blanks were also processed with the set of samples.

Extraction and cleanup of yolk samples

Pooled, spatula-homogenized yolk samples (7.0 g) were mixed with sodium sulfate and extracted with dichloromethane using pressurized fluid extraction as described previously [4]. Water was removed from extracts with sodium sulfate, and they were reduced to 10 ml in volume by evaporation using purified nitrogen. Lipid content was determined gravimetrically from a 10% subsample of the extract that was allowed to dry in a tared aluminum pan, but because this common method of determining lipids likely co-extracts other molecules such as some proteins, it is hereafter called total extractable organic content. The dry total extractable organic residue was weighed to the nearest 0.00001 g. Extracts were cleaned up with size exclusion chromatography as described in Kucklick et al. [22] followed by solid-phase extraction with alumina columns and fractionation with silica columns as described in Alava et al. [4].

Determination of POP concentrations

Both fractions (F1 and F2) from the silica column were analyzed on a gas chromatograph with dual micro-electron capture detectors (Hewlett Packard) for PCBs and certain organochlorine pesticides. Compounds were separated (2-μl injection) using two different 60-m columns (DB-5 and DB-XLB; J&W Scientific). Inlet, gas chromatograph oven, and electron capture detector parameter choices were similar to those of Kucklick et al. [22].

Both fractions of each sample were recombined (during this step we lost samples FLHI11 and FLME14), and 20 μl of each extract were injected three times onto a gas chromatograph equipped with a mass spectrometer (Agilent 6890N/5973 inert), using a programmable temperature vaporization inlet and selected ion monitoring to confirm concentrations of certain PCBs and organochlorine pesticides and to quantify the PBDEs. A 60-m DB-5MS column (J&W Scientific) was used for the first

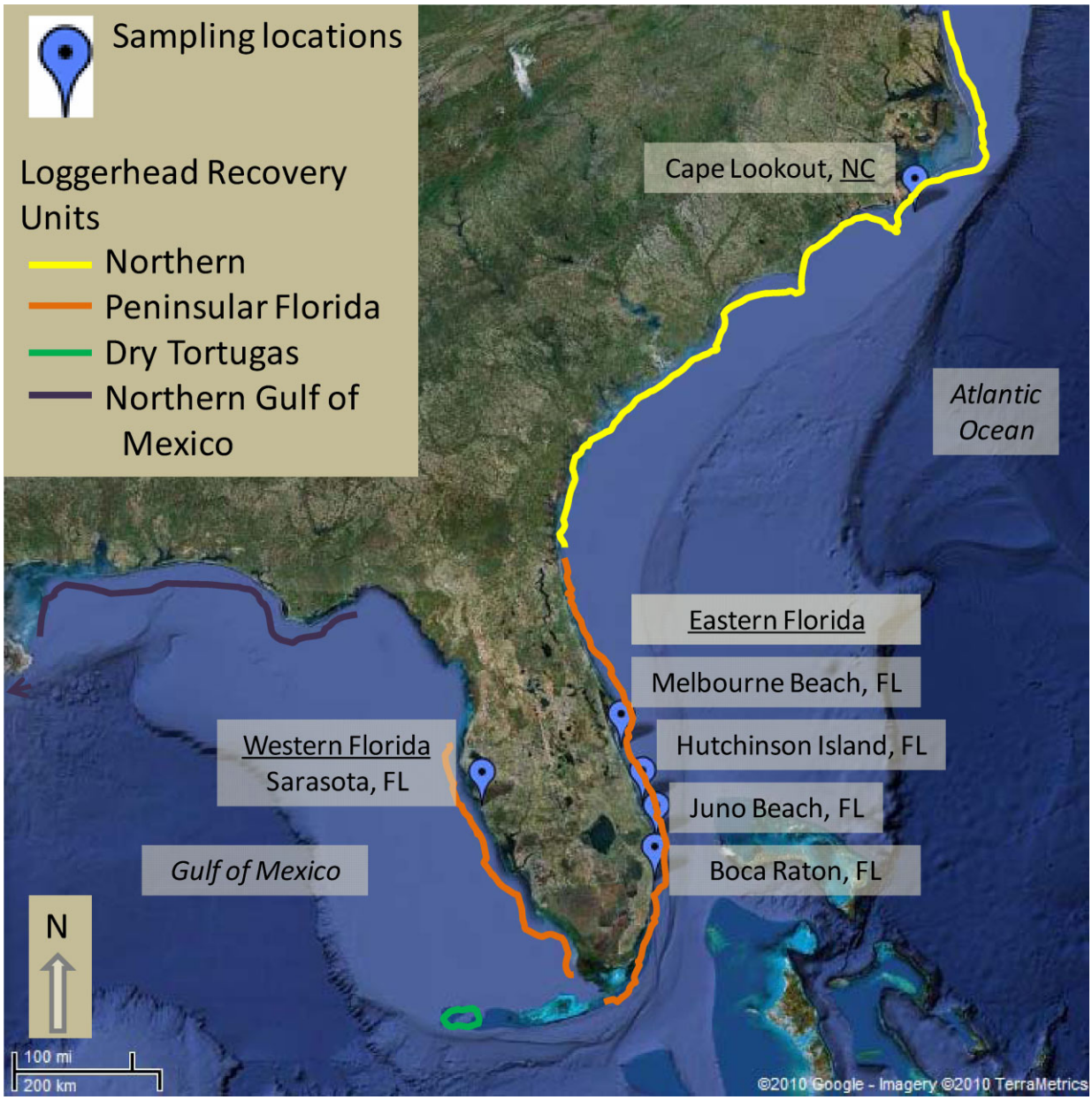

Fig. 1. Location of loggerhead sea turtle egg sampling in relation to the four identified nesting recovery units in the United States [1].

injection with electron impact mode to measure selected PCBs, DDTs, mirex, and lower-brominated PBDEs. The second injection used the same 60-m column with negative chemical ionization for toxaphenes, chlordanes, HCHs, hexachlorobenzene, endosulfans, endrin, and dieldrin. All compounds were quantified using PCB 198 as the internal standard, except for the endosulfans, for which endosulfan I-d_4 was used. The third injection used negative chemical ionization and a 15-m DB-5MS column to screen only seven samples (NCCL4, NCCL14, FLBR5, FLHI4, FLJU10, FLSA2, FLSA5) for the presence of higher-brominated PBDE congeners, which were not detectable. Inlet and instrument parameters are described elsewhere [23].

The amount of each compound was calculated using linear regressions of at least a three-point calibration curve and ratios to an internal standard compound. The minimum reporting limit (RL) for each compound was established as the nanogram in the lowest detectable calibration solution divided by the average sample mass (7 g) except for PBDEs. The RL for PBDEs was established as the average plus 3 times the standard deviation of the peak area in the blanks to account for background procedural contamination.

Statistical analysis

Concentrations were lipid-normalized by dividing the wet-mass concentration by the fraction of total extractable organic content. Only detected compounds were summed to calculate totals for a contaminant class. The ΣPCB was the sum of 49 PCB congeners. Sigma chlordane was the sum of heptachlor epoxide, oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor; ΣHCH was the sum of α-HCH, β-HCH, and γ-HCH; Σendosulfan was the sum of endosulfan I, endosulfan II, and endosulfan sulfate; ΣPBDE was the sum of 13 PBDE congeners; and Σtoxaphene was the sum of Parlars 26, 32, 50, and 62. Summary statistics were calculated using the program R version 2.11.1 (R Development Core

Team), using the NADA package, which can handle left-censored datasets or those with values less than RL as recommended by Helsel [24]. Mean, standard deviation, and median were estimated with Kaplan-Meier or regression on order models. Model choice was based on sample size and detection frequency as recommended in Helsel [24]. Regional differences in POP concentrations were determined in the following manner. Normality and homoskedasticity of raw and log-transformed data were tested using Shapiro-Wilk and Bartlett tests, respectively. For compounds that had 100% detection frequency (PCB 153, ΣPCB, 4,4′-DDE, ΣDDTs, ΣPOPs, and total extractable organic content), JMP 5.1 (SAS Institute) software was used to perform analyses of variance or Welch analyses of variance followed by Tukey-Kramer HSD multiple comparison tests ($\alpha = 0.05$). For compounds with less than 100% detection frequency, R's NADA package was used to perform either a parametric (regression by maximum likelihood estimation for left-censored data using the function *cenmle*) or nonparametric (test censored empirical cumulative distribution function differences for left-censored data using the function *cendiff*) three-group comparisons. When this test showed a significant difference among regions ($p < 0.05$) for a particular compound, then pairwise comparisons were used with the NADA functions along with a Bonferroni correction ($\alpha = 0.0167$) to determine which regions were different from each other. A principal component analysis was conducted to visualize differences among regions in the pattern of POPs. The percentage of ΣPOPs for each of the following classes were used in the principal component analysis: ΣPCB, ΣDDTs, Σchlordanes, mirex, dieldrin, ΣPBDEs, and Σtoxaphenes. Half the RL was substituted for values less than RL only for the principal component analysis, and the percentages were scaled and centered. Site differences in POP patterns were determined using analysis of variance or Wilcoxon tests followed by Tukey multiple comparison tests.

RESULTS AND DISCUSSION

Quality control

The POP concentrations measured in SRM 1946 and the loggerhead egg control material were on average 6% lower than certified or reference values in SRM 1946 and 21% different from the mean values of loggerhead egg control material determined previously [4]. These differences met our criteria for data quality.

Site differences in POP concentrations

Lipid-normalized POP concentrations were often significantly higher in NC and E FL compared with W FL (Table 2; Fig. 2). Regional differences were observed in 14 of the 15 predominant PCB congeners, except PCB 99 (Table 2), and total PCBs were significantly higher in NC and E FL than in W FL (E FL was marginally significantly higher than W FL; pairwise comparison $p = 0.023$). Some of the less predominant compounds, such as total HCHs, hexachlorobenzene, dieldrin, *cis*-chlordane, *trans*-chlordane, most DDT metabolites, PBDEs 47 and 99, and Parlar 50, were not significantly different among the three regions. Mirex was significantly higher in concentration in NC than W FL, with E FL being intermediate (Table 2, Fig. 2). The predominant chlordanes (*trans*-nonachlor, oxychlordane, and heptachlor epoxide) resulted in total chlordanes being significantly lower in W FL than in the other regions. Not surprisingly, 4,4′-DDE was the predominant DDT metabolite in all samples, and its concentrations were significantly higher in NC than W FL, with E FL having intermediate concentrations. Total PBDEs were higher in NC and E FL than W FL, and Parlar 26 was higher in NC than the FL regions. These findings generally portray an increasing gradient in POP concentrations along the southeast coast from W FL around the Florida peninsula along E FL northward to NC.

The site differences in egg concentrations of POPs suggest that the adult females nesting at these sites chose different foraging grounds; this finding is consistent with available and published tracking data compiled in Figure 3. Post-nesting satellite tracks of loggerhead turtles from Sarasota County (our W FL site) in 2005 to 2007 show foraging locations within the Gulf of Mexico or near the Bahamas, Cuba, or Dominican Republic [25]. In contrast, satellite tracks of loggerhead turtles nesting in the Northern subpopulation (near the NC site) on Bald Head Island, North Carolina (2003–2005), and Wassaw, Georgia (1997), demonstrate that they migrate along coastal Georgia to New Jersey (76%) or to eastern FL (24 %) to forage [26,27]. Based on flipper-tag return data from loggerheads nesting on Melbourne Beach (one of our E FL beaches) from 1972 to 1978 [28], we know that these turtles inhabit a wider range of locations after nesting, including the Gulf of Mexico, Bahamas, Cuba, the eastern coast of Florida, as well as the coastline from Georgia to New Jersey. The tracking methodology of this older study for E FL turtles [28] differs from the satellite methods used for the other regions. This may introduce biases because of spatial or temporal differences in observation or reporting effort, especially from distant and international locations. However, the proportions and destinations reported by Meylan and Bjorndal [28] for E FL are confirmed by recent, publicly available satellite tracks from loggerhead turtles nesting in the Archie Carr National Wildlife Refuge from Melbourne Beach to Wabasso Beach (Sea Turtle Conservancy, 2010, http://www.conserveturtles.org/satelliteturtles.php). This compiled, larger picture of postnesting migration demonstrates that loggerheads nesting in North Carolina use a very different foraging habitat, and probably associated differences in prey species, than those turtles nesting in W FL, and E FL turtles are intermediate. The tracking data therefore are consistent with contaminant concentration differences among sites, suggesting that loggerhead eggs reflect previous foraging activity of the adult females. These data also show that the Gulf of Mexico, Caribbean areas, and coastal Florida marine prey are less contaminated with these POPs than the prey in the coastal waters of Georgia to New Jersey.

Site differences in POP concentrations have been noted in loggerhead sea turtles along the U.S. East Coast in three previous studies [29–31]. Keller et al. [29] observed higher plasma concentrations of perfluorinated contaminants (PFCs) in juvenile loggerheads captured in North Carolina than from northern Florida. O'Connell et al. [30] expanded this spatial assessment of PFCs and found that plasma concentrations of the predominant PFC, perfluorooctane sulfonate, was higher in juvenile loggerheads captured in Maryland and North Carolina as compared with Cape Canaveral, Florida. Because PFCs were used for different purposes than the POPs measured in this study, and the two chemical classes have different environmental transport mechanisms, a better comparison is with the study by Ragland et al. [31]. The study by Ragland et al. found that adult male loggerhead sea turtles that migrated north and chose foraging habitats along the coastline between South Carolina and New Jersey had higher concentrations of POPs than males that remained resident at the capture site of Cape

Table 2. Persistent organic pollutant concentrations (ng/g lipid) and total extractable organic content (percent lipid) in loggerhead sea turtle pooled egg yolk samples from nests laid in three regions.[a]

	Western Florida, USA						Eastern Florida, USA						North Carolina, USA					
Compound	%>RL	*n*	Median	Mean	SE	Range	%>RL	*n*	Median	Mean	SE	Range	%>RL	*n*	Median	Mean	SE	Range
PCB 66	9	11	1.09 A	1.09	NA	<0.398–5.33	46	24	0.445 AB	2.53	1.15	<0.455–23.4	89	9	29.0 B	19.5	6.2	<0.228–39.9
PCB 99	73	11	0.888	1.81	0.68	<0.472–8.08	83	24	4.58	21.6	10.5	<0.545–243	67	9	78.2	76.8	28.3	<0.653–208
PCB 105	64	11	0.528 A	1.19	0.43	<0.318–4.45	88	24	3.21 B	20.3	10.0	<0.398–188	100	9	43.2 B	62.9	24.0	1.09–183
PCB 118	100	11	1.59 A	3.45	1.32	0.624–15.3	100	24	10.7 B	41.2	20.6	1.05–462	78	9	200 AB	165	56	<0.658–423
PCB 128	36	11	0.432 A	0.918	0.292	<0.471–3.39	88	24	2.89 B	10.2	4.5	<0.338–97.1	100	9	40.9 B	46.7	15.9	1.06–118
PCB 138 + 163	45	11	0.445 A	3.33	1.61	<0.987–16.3	88	24	12.0 B	57.8	26.9	<1.01–567	100	9	165 B	268	97	4.8–696
PCB 146	27	11	0.0583 A	0.325	0.189	<0.052–2.01	54	24	0.781 A	6.15	3.10	<0.310–70.1	89	9	27.9 B	41.7	15.2	<0.721–112
PCB 153	100	11	5.26 A	13.9	5.8	0.913–62.4	100	24	49.0 B	121	43	2.79–761	100	9	233 C	371	125	15.6–898
PCB 170	73	11	0.343 A	0.972	0.315	<0.291–2.91	100	24	3.79 B	7.07	2.61	0.313–56	100	9	12.2 C	25.2	8.2	0.894–64.1
PCB 180	64	11	0.823 A	2.73	1.09	<0.474–9.68	96	24	7.78 B	25.7	10.3	<0.773–205	100	9	35.4 B	68.0	21.2	1.99–170
PCB 183	36	11	0.253 A	0.987	0.373	<0.463–9.29	88	24	3.46 B	8.19	3.08	<0.532–64.3	89	9	13.2 C	29.6	9.8	<0.642–69.8
PCB 187	55	11	0.600 A	1.23	0.45	<0.200–5.2	83	24	3.6 B	14.7	6.8	<0.500–155	100	9	53.5 B	87.1	30.9	0.8–219
PCB 193	9	11	<1.44A	<1.49	NA	<0.304–2.73	42	24	0.789 AB	2.06	0.56	<0.279–10.7	67	9	1.40 B	3.78	1.13	<0.868–8.37
PCB 194	45	11	0.560 A	0.653	0.185	<0.239–1.66	67	24	1.41 A	2.71	0.85	<0.256–16.1	89	9	3.53 B	7.73	2.13	<0.714–18
PCB 199	40	10	0.470 A	0.720	0.165	<0.527–1.93	55	22	1.52 AB	4.31	1.32	<0.541–25.9	100	9	7.10 B	14.0	4.7	0.454–31
Total PCBs	100	11	11.4A	32.4	14.1	1.54–151	100	24	130 B	372	148	7.13–3010	100	9	1030 B	1460	493	32.9–3500
Total HCHs	27	11	0.445	0.449	0.017	<0.406–1.09	38	24	0.283	1.21	0.49	<0.426–10.4	56	9	0.956	3.15	1.39	<0.543–13.1
HCB	20	10	0.182	0.423	0.172	<0.394–1.86	16	19	0.185	0.405	0.133	<0.385–2.42	33	9	0.0409	0.678	0.450	<0.504–4.14
Mirex	45	11	0.174 A	1.04	0.53	<0.099–5.61	83	24	1.84 AB	6.78	3.78	<0.092–90.2	100	9	9.56 B	10.3	3.0	0.451–29.7
Dieldrin	100	11	3.95	5.06	1.09	1.79–14.7	88	24	6.71	10.0	1.9	<1.14–32	56	9	8.41	29.9	11.0	<1.98–76.1
cis-Chlordane	9	11	1.09	1.09	NA	<0.395–1.09	17	24	0.444	0.468	0.019	<0.86–1.85	0	9	<0.648	<0.635	NA	<0.505–<0.739
trans-Chlordane	10	10	<0.591	<0.663	NA	<0.438–1.09	14	22	0.183	0.321	0.094	<0.398–2.04	0	9	<0.669	<0.655	NA	<0.521–<0.762
cis-Nonachlor	18	11	0.126 A	0.243	0.117	<0.126–1.41	50	24	0.559 AB	1.34	0.30	<0.433–5.46	56	9	4.16B	7.26	1.61	<0.541–16.9
trans-Nonachlor	82	11	1.88 A	5.92	2.68	<0.472–30.2	96	24	15.7 B	42.8	15.1	<0.545–304	89	9	145 AB	176	68	<0.653–532
Oxychlordane	64	11	2.67 A	10.5	5.5	<0.468–57.3	92	24	19.9 B	47.8	13.0	<0.622–240	100	9	105 B	137	57	1.46–532
Heptachlor epoxide	82	11	3.05 A	4.95	1.54	<0.470–16.9	96	24	11.0 B	20.7	5.6	<0.709–115	89	9	37.3 AB	57.4	23.7	<0.651–214
Total chlordanes	91	11	5.91 A	20.8	9.6	<0.473–106	100	24	67.1 B	113	31	0.731–558	100	9	361 B	375	146	3.85–1280
2,4′-DDD	0	10	<0.573	<0.640	NA	<0.395–<1.06	0	19	<0.624	<0.747	NA	<0.386–<1.85	0	9	<0.649	<0.635	NA	<0.505–0.739
2,4′-DDE	9	11	0.562	0.562	NA	<0.396–1.06	9	23	0.00128	0.451	0.405	<0.387–9.3	0	9	<0.650	<0.636	NA	<0.506–<0.740
4,4′-DDE	100	11	12.4 A	22.7	7.0	0.811–74	100	24	55.0 AB	135	56	0.784–1030	100	9	824 B	690	250	1.89–2170
2,4′-DDT + 4,4′-DDD	45	11	0.519 AB	1.23	0.45	<0.232–4.58	19	21	0.438 A	0.709	0.253	<0.253–5.7	67	9	3.02 B	3.15	0.20	<1.06–4.3
4,4′-DDT	0	11	<0.574	<2.03	NA	<0.394–<16.0	5	21	0.597	0.597	NA	<0.385–18.4	33	9	0.138	1.83	1.10	<0.504–8.4
Total DDTs	100	11	13.9 A	23.8	7.1	2.36–74	100	24	55.0 AB	136	56	0.784–1030	100	9	829 B	694	251	4.97–2170
PBDE 47	60	10	0.664	0.766	0.077	<0.286–1.33	63	19	0.908	1.25	0.27	<0.343–4.41	89	9	1.46	2.61	0.96	<0.430–7.74
PBDE 99	40	10	0.209	0.345	0.063	<0.136–0.474	32	19	0.155	0.348	0.078	<0.142–2.28	44	9	1.38	1.66	0.13	<0.180–13.9
PBDE 100	20	10	0.243 A	0.283	0.027	<0.114–0.758	32	19	0.261 A	0.614	0.143	<0.137–1.25	67	9	2.73 B	5.23	1.61	<0.151–2.17
PBDE 153	0	10	<0.156 A	<0.177	NA	<0.035–<0.098	32	19	0.111 AB	0.335	0.094	<0.037–2.99	56	9	0.685 B	1.02	0.15	<0.046–12
PBDE 154	0	10	<0.0504 A	<0.0570	NA	<0.108–<0.304	26	19	0.031 A	0.304	0.160	<0.130–1.58	67	9	3.09 B	5.68	1.34	<0.144–1.67
Total PBDEs	60	10	0.664 A	1.08	0.20	<0.136–2.56	68	19	1.44 A	2.43	0.55	<0.163–7.82	100	9	7.80 B	13.5	4.8	0.43–37
Parlar 26	90	10	0.130 A	0.206	0.048	<0.055–0.487	95	19	0.602 B	1.06	0.29	<0.053–4.43	100	9	1.26 B	2.32	0.84	0.145–7.04
Parlar 50	80	10	0.131	0.182	0.048	<0.047–0.471	100	19	0.574	0.906	0.241	0.062–4.02	100	9	0.449	0.892	0.278	0.094–2.16
Total toxaphenes	90	10	0.270 A	0.378	0.088	<0.055–0.813	100	19	0.921 B	1.99	0.53	0.062–8.63	100	9	1.71 AB	3.22	1.11	0.238–8.95
Total extractable organics (%)	100	11	8.42	8.65	0.82	2.6–12.7	100	24	7.40	7.68	0.54	4.53–13.1	100	9	7.41	7.68	0.33	6.51–9.51

% > RL = percentage of nests with concentrations above the reporting limit; *n* = number of nests analyzed individually; SE = standard error; NA = not available; PCB = polychlorinated biphenyl; HCHs = hexachlorhexachlorocyclohexanes; HCB = hexachlorobenzene; DDD = dichlorodiphenyldichloroethane; DDE = dichlorodiphenyldichloroethylene; DDT = dichlorodiphenyltrichloroethane; PBDEs = polybrominated diphenyl ethers.

[a] Different letters after median values indicate a statistically significant difference among regions.

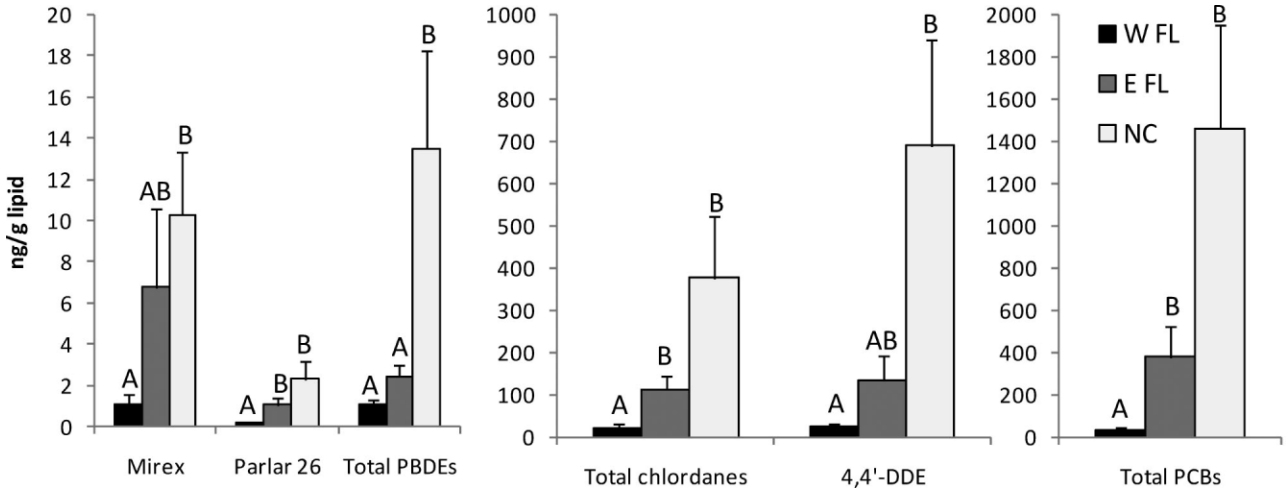

Fig. 2. Regional differences in persistent organic pollutant concentrations (ng/g lipid) in loggerhead sea turtle egg yolk samples from nests laid in three regions. Means and standard errors are shown. Different uppercase letters indicate significant differences among regions. W FL = western Florida, USA; E FL = eastern Florida, USA; NC = North Carolina, USA; PBDEs = polybrominated diphenyl ethers; 4,4′-DDE = 4,4′-dichlorodiphenyldichloroethylene; PCBs = polychlorinated biphenyls.

Canaveral, Florida. These three studies support the conclusion of the present study that sea turtles foraging farther north along the U.S. eastern seaboard have higher concentrations of POPs.

The reasons for this north–south concentration gradient likely include a combination of many factors. O'Connell et al. [30] showed that PFC concentrations in loggerhead turtles correlated with human population within the turtle capture location watershed. Thus, simply the number of people residing and using chemicals within a watershed appears to influence what is available for sea turtles and their prey to accumulate, but this logic cannot clearly explain why the W FL turtles, foraging mainly in the Gulf of Mexico, have lower contaminant concentrations, because the Mississippi River watershed drains an extremely large area with a large human population. Thus, other factors must be involved, including, but not limited to, varying types of land use in each watershed (agricultural, residential, vs industrial), atmospheric transport of POPs away from warmer southern waters toward the north, differences in sedimentation rate burying POPs as they enter the coastal regions, ocean currents transporting POPs to or from localized habitats, gradients in primary productivity, or unknown biological differences, such as different prey choices by turtles in different foraging locations.

Based on the tracking information, one might predict that the variability in contaminant concentrations would be greater in the E FL samples, because those females forage in a wider geographical range than turtles nesting in the other two regions. This hypothesis was supported for ΣPCBs and 4,4′-DDE, where the coefficients of variation were highest in the E FL samples (194 and 202%, respectively) than the other regions (101–144%). However, the highest coefficients of variation were seen in W FL for Σchlordanes and in NC for ΣPBDEs. Overall, these coefficients of variation demonstrate that POP concentrations in loggerhead samples are quite variable. This large variability is not surprising given the very large range used for foraging by each subpopulation as well as their omnivorous diet. As a species, loggerhead turtles are considered generalists, but individuals are known to specialize in a small number of prey items [32], which can contribute to large variability in POP concentrations within any group of turtles.

Site differences in POP patterns

The ΣPCBs were the dominant group of compounds in all nests, and their contribution to ΣPOPs differed significantly among regions (Fig. 4A). The ΣPCBs represented 33% on average of ΣPOPs in W FL, 49% in E FL, and 63% in NC. The ΣDDTs and Σchlordanes were the next highest class of contaminant measured in all three regions, followed by dieldrin. Mirex, ΣPBDEs, and Σtoxaphenes made up minor contributions (1.2%, 1.6%, 0.6%, respectively) of ΣPOPs. In addition to ΣPCBs, significant regional differences were observed in the proportion of ΣPOPs represented by dieldrin (higher in W FL) and Σchlordanes (higher in E FL).

The principal component analysis resulted in the first two principal components (PCs) accounting for 57% (35% for PC1 and 22% for PC2) of the variation in POP patterns and large overlap among the regions on the PC score scatterplot (Fig. 4B). North Carolina and E FL overlap completely on this score plot, as does E FL with W FL, but NC and W FL separate somewhat along both PC1 and PC2, revealing that the two most distant locations differ the most in POP patterns. High loadings for PC1 came from ΣPCBs and dieldrin, which is not surprising because dieldrin made up a large percentage of the difference in ΣPOP contributions seen between NC and W FL. In fact, W FL had higher average contributions of most pesticides (mirex, dieldrin, ΣDDTs, and Σtoxaphenes) than E FL and NC, suggesting that, relative to PCBs, the Gulf of Mexico is more contaminated with pesticides than the western Atlantic Ocean. This finding is not surprising when one considers the large agricultural watersheds (e.g., the Mississippi River) draining into the Gulf of Mexico, resulting in a higher proportion of legacy pesticide inputs relative to more industrial PCB compounds.

A more detailed look at the PCB congener patterns revealed some regional differences (Fig. 5A). The overall PCB pattern observed is typical for biological samples, with congeners 99, 105, 118, 138 + 163, 153, 170, 180, and 187 dominating. Interestingly, the contribution of PCB 153 was significantly higher in W FL than NC, with E FL being intermediate. The contributions of PCBs 66 and 146 to ΣPCBs were higher in NC than the other regions, and contributions of PCBs 128 and

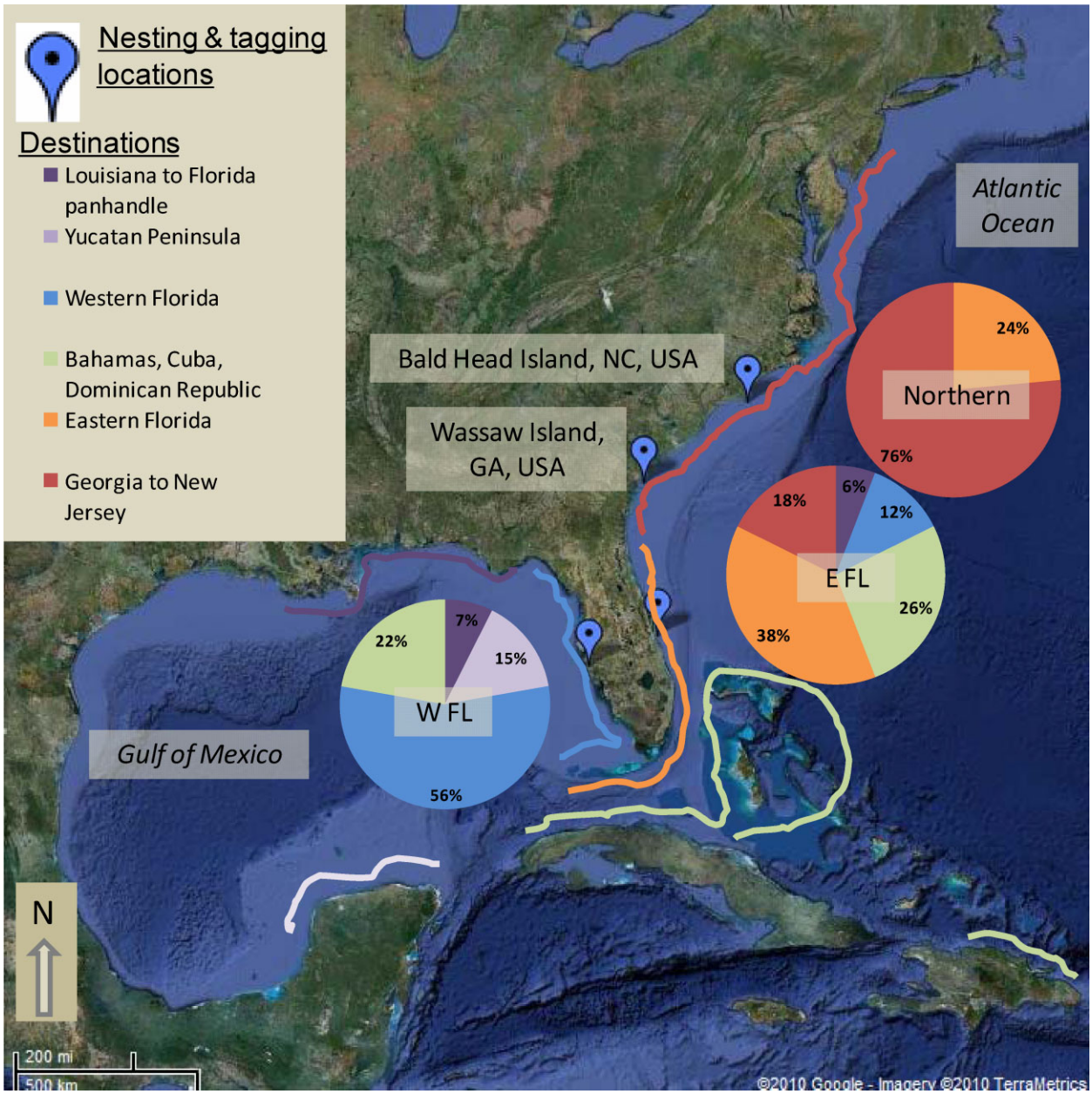

Fig. 3. Post-nesting migration tracking data available for loggerhead sea turtles nesting near the sampling locations of the current study. Pie charts indicate the percentage of nesting loggerhead turtles that migrated to the different color-coded destinations. General destinations are shown as drawn lines. Satellite tracking data were compiled for the Northern Recovery Unit from Bald Head Island, North Carolina, and Wassaw Island, Georgia, nesting beaches [26,27]. Tag return data from Melbourne Beach in eastern Florida came from Meylan and Bjorndal [28]. Satellite tracking data from Sarasota County in western Florida came from Girard et al. [25]. W FL = western Florida, USA; E FL = eastern Florida, USA; NC = North Carolina, USA.

138 + 163 were higher in W FL. The reason for these pattern differences is unknown, but they could be attributable to different PCB technical mixtures dominating the food webs and foraging habitats of these females.

The PBDE pattern differences might be more interesting than those of the PCBs (Fig. 5B). The W FL samples displayed a typical PBDE pattern, with PBDE 47 dominating, followed by PBDEs 99 and 100, and lesser contributions from PBDEs 153 and 154. In contrast, NC samples showed nearly equal contributions of PBDEs 47, 100, and 154 on average, and the E FL samples showed an intermediate pattern. Statistically, the contribution of PBDE 154 to ΣPBDEs was higher in NC samples than the other regions. Hites [33] reviewed the literature of PBDE concentrations in a wide variety of biological samples mostly showing the typical pattern that we observed in W FL. Atypical patterns, similar to the one in the NC samples, have been noted recently in plasma samples from subadult loggerhead turtles from North Carolina (Carlson, 2006, Master's thesis, College of Charleston, Charleston, SC, USA), adult male loggerhead turtles that forage along the U.S. eastern coast from South Carolina to New Jersey [31], and other reptilian species, including freshwater turtles (*Sternotherus odoratus* and *Trachemys scripta troosti*) from Tennessee [23] and diamondback terrapins (*Malaclemys terrapin*) from New Jersey [34]. This atypical pattern is not species specific, because loggerhead eggs from W FL have the typical pattern, but instead it seems to be geographically specific to latitudes of 30°N to 40°N in North America. North of 40°N, snapping turtles (*Chelydra serpentina*) again show the typical PBDE pattern, with PBDE 47 dominating [35]. This spatial difference could be attributable to releases

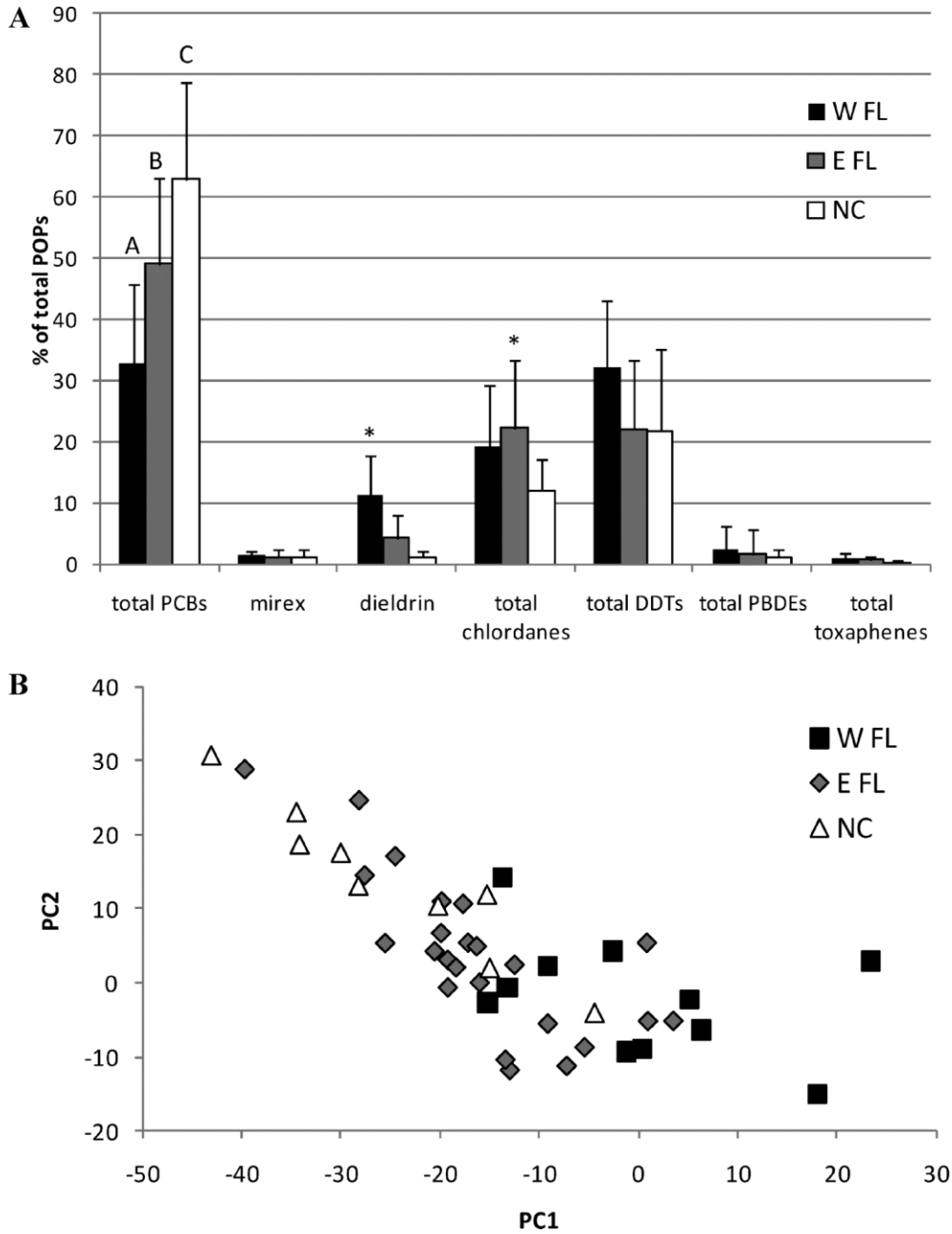

Fig. 4. Persistent organic pollutant (POP) patterns in loggerhead sea turtle egg yolk samples from nests laid in three regions. W FL = western Florida, USA; E FL = eastern Florida, USA; NC = North Carolina, USA. (**A**) Summed contaminant classes as a total of all POPs; data are mean ± 1 standard deviation. Different uppercase letters above columns indicate significant differences among sites for that compound. An asterisk (*) above a column indicates significant difference between that site and both others for that compound. Lack of letters or asterisk indicates no significant site differences for that compound. PCBs = polychlorinated biphenyls; DDTs = dichlorodiphenyltrichloroethane-related compounds; PBDEs = polybrominated diphenyl ethers. (**B**) Scatterplot of the first two principal component (PC) scores.

of different PBDE formulations, although other taxa, including piscivorous birds and freshwater fish, within 30 to 40°N, demonstrate the typical pattern of PBDE 47 predominating [36,37]. Another possible reason is different metabolic breakdown or elimination of congeners in reptiles inhabiting different climates; future studies should investigate this latter possibility.

Comparison of POP concentrations with other studies

Only one previous study of loggerhead egg POP concentrations is available to compare with our lipid-normalized concentrations. The average ΣPCB concentrations in loggerhead eggs from South Carolina (1,188 ng/g lipid) [6] were consistent with the spatial gradient we observed. Those concentrations were much higher than E FL and less than NC. Eggs from the leatherback sea turtle (*Dermochelys coriacea*) nesting in E FL were measured for POPs recently [13], and they have lower average concentrations of certain POP classes compared with the E FL loggerheads (mean ± standard deviation in ng/g lipid for the leatherback eggs were 171 ± 150 ΣPCBs, 1.69 ± 0.12 mirex, 46.0 ± 33.4 Σchlordanes, 37.9 ± 20.3 ΣDDTs), but similar concentrations of dieldrin (10.8 ± 6.8) and Σtoxaphenes (1.49 ± 0.63) and higher concentrations of ΣPBDEs (17.1 ± 12.6). The first four POP classes follow an

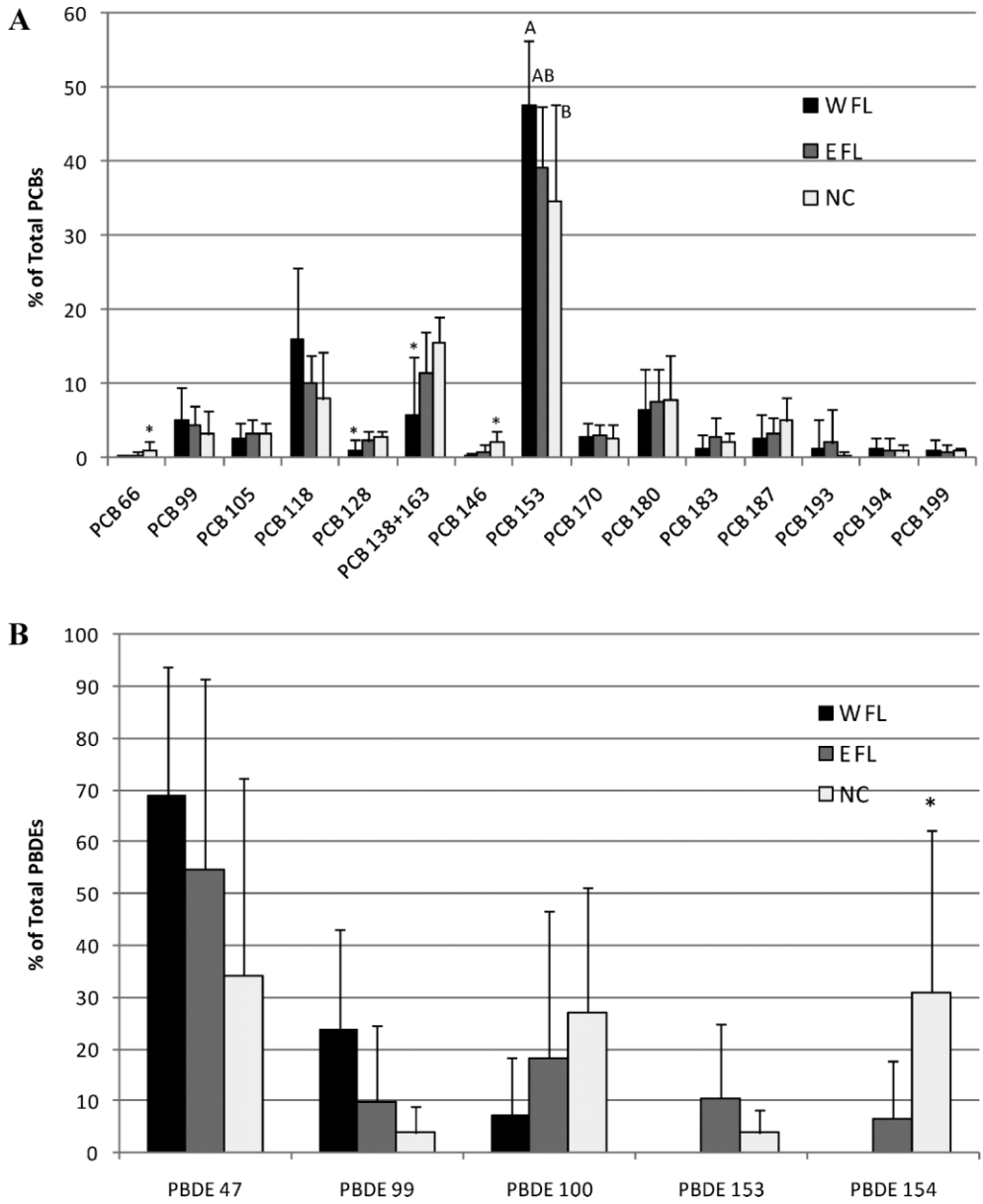

Fig. 5. PCB (**A**) and PBDE (**B**) patterns in loggerhead sea turtle egg yolk samples from nests laid in three regions. W FL = western Florida, USA; E FL = eastern Florida, USA; NC = North Carolina, USA; data are mean ± 1 standard deviation. Different uppercase letters above columns indicate significant differences among sites for that compound. An asterisk (*) above a column indicates significant difference between that site and both others for that compound. Lack of letters or asterisk indicates no significant site differences for that compound. Only congeners with more than 1% of total for any region are shown. PCB = polychlorinated biphenyl; PBDE = polybrominated diphenyl ether.

expected trend based on trophic differences, because the gelatinous zooplankton-consuming leatherback sea turtle feeds lower on the food chain than the omnivorous loggerhead. However, the latter three comparisons (dieldrin, Σtoxaphenes, ΣPBDEs) are surprising and likely attributable to differing foraging locales, with the leatherback inhabiting water both much further north than the loggerhead [38] and much deeper.

A previous review has noted that sea turtle ΣDDT concentrations can be orders of magnitude lower than concentrations measured in other marine wildlife, such as some marine mammals and seabird species [39]. This holds true for most POPs; for example, maximum PBDE 47 concentrations measured in the loggerhead eggs was 7.74 ng/g lipid, which is much lower than average concentrations found in seabird eggs (≈1,000 ng/g lipid) [33]. Although this puts concerns over toxicity into perspective, toxic thresholds are essentially unknown for sea turtles. Without knowing how sensitive developing loggerhead sea turtles are specifically to these compounds, determining the risk of POPs to embryonic development, maturation, and to species survival is not possible. A future study will report on correlations between the concentrations measured here and measures of health, fitness, and mortality, including hatching success, developmental abnormality rates, growth rates, mortality within the first six months post-hatching, and sex ratios of the resulting hatchlings from the same nests (Keller et al., unpublished data).

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