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FORTY-SEVEN DAYS OF DECAY DOES NOT CHANGE PERSISTENT ORGANIC POLLUTANT LEVELS IN LOGGERHEAD SEA TURTLE EGGS

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Abstract—Reptile and bird eggs are priority samples for specimen banking programs that assess spatial and temporal trends of environmental contaminants. From endangered species, such as sea turtles, nonlethal sampling is required (e.g., unhatched eggs collected postemergence). Previous contaminant monitoring studies have used unhatched sea turtle eggs, but no study has tested whether their concentrations represent levels found in fresh eggs (e.g., eggs collected within 24 h of oviposition). The author analyzed three fresh eggs from different nest depths and up to three unhatched eggs from 10 loggerhead sea turtle (*Caretta caretta*) nests in South Carolina, USA, for a suite of persistent organic pollutants (POPs). Lipid-normalized POP concentrations were not significantly different ($p > 0.05$) between fresh and unhatched eggs or among different depths from the same nest. The POP concentrations in loggerhead eggs from South Carolina were higher than previously measured concentrations in eggs from Florida and slightly lower than concentrations in eggs from North Carolina. This pattern agrees with previously observed trends of increasing POP concentrations in loggerhead turtles inhabiting northern latitudes along the U.S. East Coast. Contaminant profiles are discussed, including a higher chlorinated pattern of polychlorinated biphenyls possibly associated with a Superfund site in nearby Brunswick, Georgia, USA, and unusual polybrominated diphenylether patterns seen in this and previous sea turtle studies. Concentrations correlated with one of eight measurements of reproductive success; levels were negatively correlated with egg mass ($p < 0.05$), which may have implications for hatchling fitness. The present study suggests that unhatched eggs can be used for POP-monitoring projects. Environ. Toxicol. Chem. 2013;32:747–756.

Keywords—Marine turtle Reptile egg Environmental contaminant Environmental specimen banking Nonlethal sampling

INTRODUCTION

Bird and reptile eggs are preferred samples for monitoring environmental organic contaminants [1–3] because they are easily collected and contain a lipid-rich yolk where persistent organic pollutants (POPs) accumulate. Concentrations of POPs in eggs represent the contamination level and pattern of the maternal adult foraging areas [2,4,5] as well as the internal environment for embryonic development. For these reasons, many environmental specimen banks archive eggs for long-term monitoring projects or for retrospective research. For example, a set of guillemot (*Uria algae*) eggs archived yearly from St. Karlsö in the Baltic Sea provided data for one of the first temporal trend studies of polybrominated diphenyl ethers (PBDEs) [6]. That study demonstrated a significant temporal increase of PBDEs from 1969 through 1990 [6], a finding that was instrumental in the regulation and discontinued use of certain PBDE formulations in many countries [7].

Environmental specimen bank managers must consider many biological factors when deciding how to sample certain species. Successful programs must be able to sample an adequate number of individuals per year (or at the intended frequency) for a robust statistical design while minimizing the negative impact on the species of interest. Trade-offs are often made that might bias sampling toward higher or lower contaminated samples, and these biases must be taken into consideration when interpreting results [8].

A sea turtle specimen bank is currently being developed as part of the existing Marine Environmental Specimen Bank at the National Institute of Standards and Technology (NIST) [9]. This project is titled Biological and Environmental Monitoring and Archival of Sea Turtle Tissues (BEMAST), and eggs are a priority sample. Inclusion of eggs into a sea turtle specimen bank for environmental contaminant monitoring is justified by recent sea turtle research [10]. We have recently learned that (1) POPs are maternally transferred into the egg yolk of sea turtles [11,12]; (2) POP concentrations in eggs represent contamination in the maternal adult foraging areas [2]; (3) POP concentrations, specifically in the yolk compartment, change through embryonic development because yolk lipids concentrate as the embryo grows [13]; and (4) there is low variability in POP concentrations among eggs within a clutch [12,13].

All sea turtle species in the United States are listed in the Endangered Species Act as either threatened or endangered, which restricts sampling. Most sea turtle species lay 100 or more eggs per clutch [14], thus, taking a single fresh egg (e.g., egg collected within 24 h of oviposition) per nest might not threaten a stable or growing population. However, population trajectories of certain sea turtle populations are declining or are unknown [15,16]. Considering this, lethal sampling of a fresh egg is not warranted, especially since several unhatched (addled) eggs could be collected postemergence. It is common to find several eggs per nest that failed to hatch [14], which can be used as nonlethal samples for environmental monitoring or specimen banking.

Aside from nonlethal sampling of maternal blood, another nonlethal sampling technique has been described for POP monitoring using loggerhead (*Caretta caretta*) sea turtle eggs [17]. The chorioallantoic membrane allows gas exchange and waste storage for the embryo and is left behind after the

All Supplemental Data may be found in the online version of this article.

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hatchling emerges. Sampling the chorioallantoic membrane is problematic because (1) it represents only one compartment of the egg and therefore has a different polychlorinated biphenyl (PCB) concentration from the contents of the whole egg [17]; (2) it is available from only late-stage or hatched eggs; and (3) it is greatly disturbed and mixed with sand during hatchling emergence. For these reasons, the NIST specimen bank is proposing to archive unhatched eggs that contain either no embryo (infertile) or early arrested-development embryos. The entire egg contents minus the shell will be homogenized, which will capture all internal compartments, alleviating the need to correct for unequal distribution of contaminants. Also, by selecting eggs with no or early arrested development, the duration of decomposition in unhatched eggs is better standardized and metabolism by embryonic organs is avoided or minimized.

The use of addled eggs is common in bird contaminant-monitoring studies [8], and several studies have reported POP concentrations in unhatched sea turtle eggs [2,11,13,18]. However, no study has compared POP concentrations in fresh eggs with concentrations in eggs that failed to hatch from the same nests for any sea turtle or bird species. During nest incubation (42–91 d) for loggerhead turtles [14], embryonic development, decomposition, bacterial metabolism, dehydration, and exposure to sand, rainwater, groundwater, and overwash by the ocean could all potentially alter the contaminant concentrations originally contained inside the egg. An additional, yet unlikely, bias could be egg position within the oviduct and, therefore, within the clutch. Bishop et al. [19] observed that the first few eggs released from the oviduct of snapping turtles (*Chelydra serpentina*) tended to have higher POP concentrations than those released last, although the differences were not statistically significant. A difference is not expected in sea turtles based on the physiology of follicle development, as all follicles for a nesting season are developed many months prior to nesting [14], and since studies have shown small intraclutch variability in POP concentrations (relative standard deviations of 14% or less) [12,13]. Regardless, the variability of POP concentrations related to the egg position within a clutch has also not been tested in any sea turtle species.

The first objective of the present study was to determine if concentrations of selected PCBs, organochlorine pesticides, PBDEs, and hexabromocyclododecanes (HBCDs) are significantly different between fresh and unhatched loggerhead turtle eggs. The second objective was to determine if POP concentrations are significantly different among eggs deposited at different nest depths. A difference would decrease the validity of using unhatched eggs for a specimen bank because the position of unhatched eggs would be unknown after hatchlings dig their way up through the sand. Answering these questions is necessary before establishing a specimen bank of sea turtle eggs.

In addition, POP concentrations and patterns measured in the present study were compared to previously published data [2,17] for a better understanding of spatial and temporal trends. The eggs were collected from Botany Bay Island Plantation, South Carolina, USA, which is the closest nesting beach to a Georgia Superfund site monitored to date. Although these nesting females forage on a wide assortment of marine invertebrates [20] in areas distant from the nesting beach [21–23], it was interesting to evaluate exposure to the unusual, highly chlorinated PCB pattern known to contaminate the Brunswick, Georgia, USA, area [24,25]. Likewise, unusual patterns of PBDE accumulation have been noted in loggerhead eggs from North Carolina but not from Florida [2]; thus, these South Carolina eggs were assessed for similarities to other regions. Furthermore, to address the adverse effects of these POP exposures, correlative relationships were assessed between POP concentrations and reproductive success variables (e.g., clutch count, incubation duration, a nest success index, egg mass, egg lipid content, and success measures of embryonic development, hatching, and emergence). Measured concentrations are discussed in the context of POP concentrations that were suspected to cause endocrine disruption in wild snapping turtles and American alligators (*Alligator mississippiensis*) [26,27].

MATERIALS AND METHODS

Sample collection and processing

Eggs were collected from 10 loggerhead turtle nests laid between July 12 and 20, 2010, at Botany Bay Plantation on Edisto Island, South Carolina, USA. Each nest was confirmed to be from a different female by maternal nuclear DNA genetic identification (B. Shamblin and C. Nairn, University of Georgia, Athens, GA, USA, personal communication). Three fresh eggs from different depths within each nest (top of clutch, representing the last few eggs deposited from the oviduct of the female; center of clutch; and bottom of clutch) were collected within 12 h of oviposition while nests were being relocated to higher ground. Eggs were placed in plastic bags, rinsed with Millipore water to remove sand, dried with a cleanroom wiper, and opened carefully so that all of the egg contents fell into an acid and solvent–prerinsed glass jar with a Teflon-coated lid. Samples were frozen initially at −10°C and later transferred to −80°C for long-term storage. The number of eggs in each clutch (clutch count) was determined during relocation. Nests were marked with a sign, caged for predator exclusion, and monitored daily for overwash and hatchling emergence to determine incubation duration. After a 72-h postemergence waiting period, all 10 nests were inventoried to assess reproductive success. The numbers of hatched eggshells, dead hatchlings, live hatchlings, and unhatched (including dead pipped) eggs were determined. Unhatched eggs from a single nest were combined into a plastic bag, frozen at −10°C, and later transferred to −80°C. Unhatched eggs were thawed and opened as described above into prerinsed glass jars. Egg contents were weighed and crudely staged as no development or early, middle, or late embryonic development, as described in Alava et al. [13]. Up to three unhatched eggs were selected from each nest that externally appeared to be the freshest (round and plump eggs with a white intact shell were preferred) and internally contained either no embryo or early arrested development. Embryonic development success was defined as the percentage of eggs that developed to late stage or further and calculated as

$$100\% - \left(\frac{\textit{unhatched eggs with no, early, or middle development}}{\textit{total eggs laid} - \textit{eggs taken or broken}} \times 100\right)$$

Hatch success was calculated as

$$100\% - \left(\frac{\textit{unhatched} + \textit{dead pipped eggs}}{\textit{total eggs laid} - \textit{eggs taken or broken}} \times 100\right)$$

Emergence success was calculated as

$$100\% - \left(\frac{\textit{unhatched} + \textit{dead pipped eggs} + \textit{dead and live hatchlings}}{\textit{total eggs laid} - \textit{eggs taken or broken}} \times 100\right)$$

While these are not the traditional equations used for hatch and emergence success [28], they were chosen because they use

the most accurate counting methods (egg counts at relocation and unhatched eggs rather than egg shell counts at inventory) and represent the inherent fitness of the embryos rather than external factors of egg loss (research and relocation effects). Two nests were partially depredated by raccoons, a large external factor of egg loss, so the three reproductive success variables were not calculated for those nests. To calculate a nest success index, each nest was ranked (1 = *worst*, 8 = *best*) three times using embryonic development, hatch, and emergence success. Then, the three ranks per nest were summed for an overall nest success value.

Extraction and POP quantification

Detailed analytical methods are described in Supplemental Data. Briefly, egg contents, three blanks, a six-point calibration curve containing a mixture of the targeted POPs, three replicates of NIST Standard Reference Material 1946 Lake Superior Fish Tissue, and three replicates of an in-house pooled loggerhead turtle egg control material (called Cc comp) with previously published values [13] were extracted with dichloromethane using pressurized fluid extraction after being spiked gravimetrically with an internal standard solution containing 28 ^{13}C-labeled POPs. The percentage of total extractable organics (traditionally called lipid content) was determined gravimetrically. Extracts were cleaned up using size exclusion chromatography as well as alumina and acidified silica columns. Fraction 1 from acidified silica columns was analyzed using gas chromatography mass spectrometry for quantification of pentachlorobenzene, hexachlorobenzene, α-hexachlorocyclohexane (HCH), β-HCH, γ-HCH, six dichlorodiphenyltrichloroethane (DDT)–related compounds, oxychlordane, *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, heptachlor, octachlorostyrene, mirex, and selected PCB and PBDE congeners. Selected congeners included those that were predominant in previously analyzed loggerhead turtle eggs [2] (PCBs 153 + 132, 118, 138 + 163, 180, 99, 187, 170, 105, 128, and 193; PBDEs 47, 99, 100, 153, and 154) as well as PCBs 199 and 206 because they are indictors of Aroclor 1268, a highly chlorinated PCB mixture contaminating an Environmental Protection Agency Superfund site in Brunswick, Georgia, USA [24,25], within 300 km of Botany Bay Plantation, South Carolina, USA. Fraction-2 extracts were analyzed by liquid chromatography tandem mass spectrometry for α-HBCD, β-HBCD, and γ-HBCD. Reporting limits (RLs) were determined as described previously [29] and were typically <0.1 ng/g wet mass or <2 ng/g lipid (Supplemental Data, Table S1). When summing compound classes, concentrations below the RL were set to zero.

Statistical methods

Statistical tests were performed using JMP 10.0 software (SAS Institute) when all concentrations were >RLs or as suggested by Helsel [30] using the NADA package for handling data with nondetects (left censored data) in the open-source R software package. Unless otherwise specified, $p < 0.05$ was considered significant. An analysis of variance with repeated measures was used in JMP with lipid-normalized, log-transformed POP concentrations to compare fresh eggs from the top, middle, and bottom of nests and to compare fresh eggs to unhatched eggs. For compounds with <100% detection (∑PBDEs and α-HBCD), these tests were performed using only nests that had detectable concentrations. Concentrations in all eggs from a single nest were used to calculate each nest's mean concentration. Mean concentrations for each nest were used to calculate mean, median, and variance across all 10 nests. When some eggs or nests had concentrations <RL, Kaplan-Meier or regression on order models in R, NADA, were chosen to estimate these values. Differences between the South Carolina loggerhead turtle egg POP concentrations in the present study and loggerhead egg concentrations from other regions previously published by Alava et al. [2] were determined in NADA with an analysis of variance followed by pairwise Wilcoxon tests with a Bonferroni correction to the alpha value ($p < 0.017$ was considered significant for the spatial comparisons). Alava et al. [2] measured a larger number of PCB and PBDE congeners than the present study; therefore, new compound sums and ratios were calculated from their original data for the best comparisons to the present study. Kendall's tau correlations were used to determine relationships between lipid-normalized POP concentrations and reproductive success variables. For POPs with <100% detection (*cis*-chlordane, PBDEs 100 and 153, and ∑PBDEs), a Kendall's tau correlation for left-censored data was used. To account for the multiple correlations assessed (eight variables per compound), a Bonferroni correction was applied to the alpha value ($p < 0.006$ was considered statistically significant); however, for this preliminary assessment, $p < 0.05$ was considered marginally significant.

RESULTS

The mean (± standard deviation) clutch count for the 10 nests was 90 ± 20 eggs, with a mean incubation duration of 47 ± 2 d (Table 1). None of these nests were overwashed. A total of 178 unhatched eggs were available for developmental staging and selection for contaminant analysis. The number of unhatched eggs ranged from 0 to 55 per nest, with a mean of 18 eggs per nest. Mean embryonic development, hatch, and emergence success measures were 76, 73, and 43%, respectively. Most unhatched eggs ($n = 155$) contained either no or early arrested embryonic development. Embryos of later developmental stages ($n = 10$) were seen in only five nests. Three of these five nests had one to three dead pipped embryos (10-BTB-154, 10-BTB-153, and 10-BTB-156), and two of these five nests had one or two late-stage embryos (10-BTB-165 and 10-BTB-163; Table 1).

Fifty-four eggs were selected for contaminant analyses, but POP data from two of these were excluded because of problems during analysis (Table 1). Selected samples included three fresh eggs from each of the 10 nests plus zero (from nests where all eggs hatched) to three unhatched eggs from these same nests postemergence. The selected unhatched eggs were typically in good condition, with a few being slightly decomposed or showing bacteria or fungus evidenced by pink or black coloration. Contents from the selected unhatched eggs often weighed a few grams less than contents from fresh eggs, most likely because of dehydration (Table 1). Unhatched eggs had higher average total extractable organics content than fresh eggs, and although this was not statistically significant ($p = 0.0694$), it indicated the importance of lipid normalizing the POP concentrations.

The total extractable organics and POP concentrations measured in the control materials did not deviate from certified or previously measured values. The percentage of difference averaged across all compounds was 9.3 and −2.2% for NIST Standard Reference Material 1946 and the loggerhead turtle egg composite, respectively.

Table 1. Reproductive success variables and description of eggs chosen for analysis of persistent organic pollutants

Nest ID	Clutch count	Incubation duration (d)	# of unhatched or dead pipped eggs staged	Embryonic development success (%)	Hatch success (%)	Emergence success (%)	Nest success index	Sample type for POPs	# of eggs analyzed for POPs	Description of unhatched eggs analyzed for POPs	Mean egg contents (g)	SD egg contents (g)	Mean TEO (%)	SD TEO (%)
10-BTB-145	88	48	13	85	84	2	12	Fresh	3		27	3	5.39	0.90
								Unhatched	3	All 3: no develop; yellow; good condition	25	4	5.43	0.47
10-BTB-146	66	48	2	97	95	85	21	Fresh	3		32	1	7.12	0.67
								Unhatched	2	Both: no develop; yellow with orange and pink; moderate decomposition and fungus present	26	1	7.78	0.11
10-BTB-150	108	47	0[a]	NA	NA	NA	NA	Fresh	2[b]		37	1	6.34	0.42
								Unhatched	0[a]					
10-BTB-151	121	47	30	75	73	59	14	Fresh	3		32	2	5.03	0.28
								Unhatched	3	All 3: no develop; yellow (one egg had a black spot); good condition	32	2	4.45	0.27
10-BTB-153	98	49	3	98	97	97	24	Fresh	3		26	1	5.63	0.55
								Unhatched	2	1: no develop; yellow; good condition; 1: early embryo, yellow; shell damaged & fungus present	16	5	10.70	4.24
10-BTB-154	53	49	3	96	94	42	16	Fresh	3		35	1	6.10	0.41
								Unhatched	2	Both: no develop; yellow/cream; slight decomposition	26	1	8.13	1.21
10-BTB-156	98	47	8	NA	NA	NA	NA	Fresh	2 or 3[c]		35	5	5.91	0.33
								Unhatched	3	All 3: no develop; yellow; good condition but one egg was very liquidy	33	2	5.26	0.93
10-BTB-162	98	46	29	69	68	0	7	Fresh	3		38	2	5.85	0.20
								Unhatched	3	1: no develop; yellow; good condition; 2: early embryo; yellow/pink; very slight decomposition	37	2	6.07	0.56
10-BTB-163	81	45	55	31	22	14	5	Fresh	3		29	2	6.00	0.46
								Unhatched	3	All 3: no develop; yellow; good condition	29	1	6.49	0.39
10-BTB-165	88	43	35	58	52	46	9	Fresh	3		33	0	5.47	0.36
								Unhatched	3	All 3: no develop; yellow; good condition	34	1	5.06	0.44

[a] No unhatched eggs available because of raccoon depredation.
[b] Only top and middle fresh eggs were reported. POP and TEO data for 150-bottom was excluded because of poor TEO recovery and inaccurate lipid normalization of POPs.
[c] Three eggs analyzed for hexabromocyclododecanes, but F1 extract of 156-middle was lost prior to gas chromatography injection.
POP = persistent organic pollutant; SD = standard deviation; TEO = total extractable organics; NA = not available (nest was partially depredated by raccoons).

The following compounds were not detected above the RL in any South Carolina sample: PBDE 47, PBDE 99, pentachlorobenzene, hexachlorobenzene, α-HCH, β-HCH, γ-HCH, 4,4'-DDT, heptachlor, octachlorostyrene, β-HBCD, and γ-HBCD. Four DDT-related compounds (2,4'-dichlorodiphenyldichloroethylene, 2,4'- and 4,4'-dichlorodiphenyldichlorethane, and 2,4'-DDT) were detected in only one egg at concentrations very near the RL. Concentrations of POPs with more frequent detection are shown in Figure 1 for each fresh egg and the mean of the unhatched eggs. No significant difference was observed in any POP concentration among fresh eggs at different depths within a nest ($p \geq 0.25$) or between fresh eggs and unhatched eggs of the same nest ($p \geq 0.10$). Thus, POP concentrations in all eggs from the same nest could be combined to determine mean POP concentrations for each nest.

The POP concentrations in this study were significantly greater than concentrations previously measured [2] in loggerhead turtle eggs from western and eastern Florida but not significantly different from concentrations in eggs from North Carolina (Table 2). Statistical power was enough to detect these differences, even though the sample sizes were small from each location ($n = 10$ nests from South Carolina). The ratio of PCB 206 to PCB $153 + 132$, an indicator of exposure to Aroclor 1268, was highest in eggs from South Carolina but not significantly different from the other regions (Table 2, Fig. 2). Three South Carolina nests had ratios higher than 0.1, with one of these nests (10-BTB-154) having a ratio of 0.393. A ratio above 0.1 was seen in only one nest from the other locations. The PBDE congener profile seen in these eggs was unusual compared to most other wildlife [31]. Amounts of PBDEs 47 and 99 were below RL, and PBDEs 100 and 154 were predominant, making the South Carolina profile different from those of the other three regions (Fig. 3).

None of the eight reproductive success variables significantly correlated to concentrations of any POP, using $p < 0.006$ as a strict threshold for multiple correlations; however, one variable, the mass of fresh eggs, correlated in a marginally significant manner ($p < 0.05$) to several POP concentrations (see Supplemental Data, Table S2 for correlation coefficients and p values). The variables that were not significantly correlated to POPs using either p-value threshold were clutch count, incubation duration, embryonic development success, hatch success, emergence success, nest success index, and total extractable organics content. Nests with higher concentrations of PCB 138, *cis*-nonachlor, PBDEs 100 and 153, and total PBDEs had lower fresh egg masses (Supplemental Data, Table S2; Fig. 4; $p < 0.05$). Nests with higher ratios of PCBs 206:$(153 + 132)$ had lower egg masses (Supplemental Data, Table S2; Fig. 4; $p < 0.05$).

DISCUSSION

Variability within a nest was minimal (mean within-nest relative standard deviation averaged across all compound

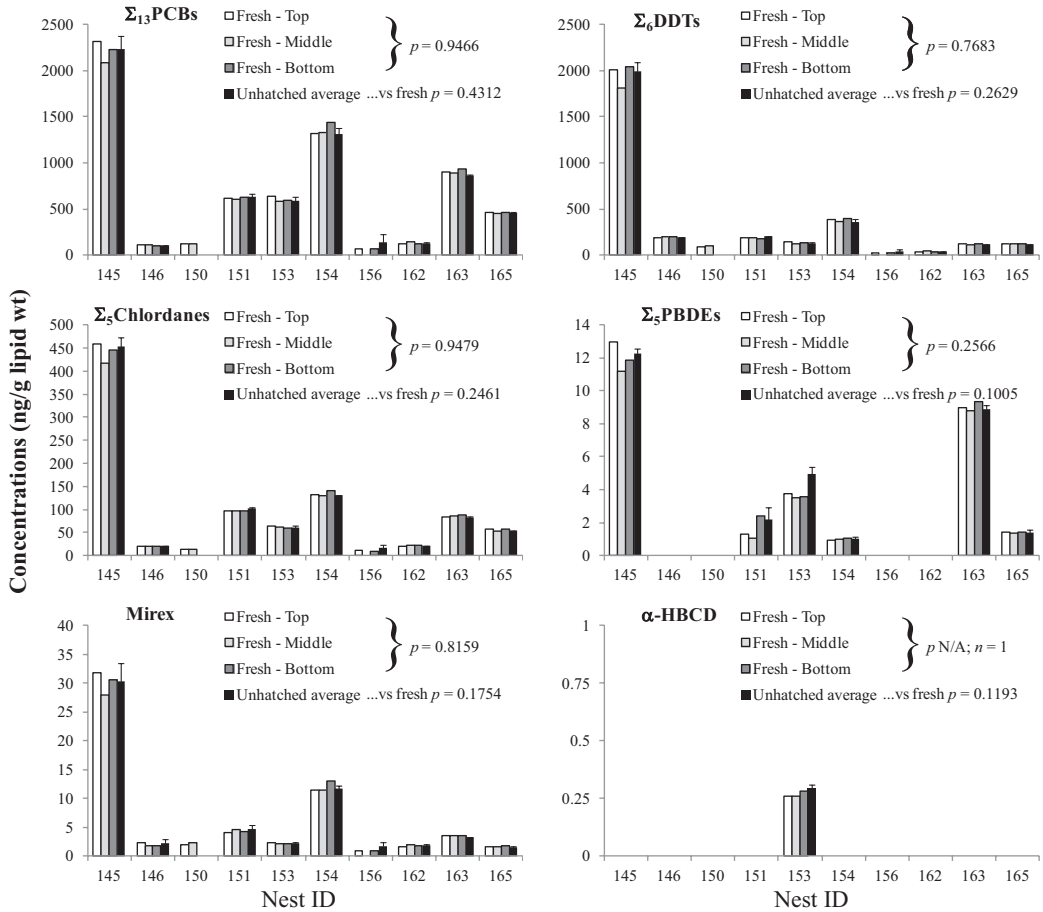

Fig. 1. Comparison of persistent organic pollutant concentrations (mass fractions) in unhatched eggs (mean and one standard deviation) and fresh eggs from the top, middle, and bottom of 10 loggerhead turtle nests laid on Botany Bay Plantation, Edisto Island, South Carolina, USA. The p values are from repeated measures analysis of variance. Missing columns indicate that either the sample was below the reporting limit or the data were excluded because of sample-processing problems.

Table 2. Summary of persistent organic pollutant concentrations (mass fractions as ng/g lipid wt) in egg contents of 10 loggerhead turtle nests from Botany Bay Plantation, Edisto Island, South Carolina, USA, compared to previously published concentrations in egg yolks from nests laid in different regions along the southeastern coast of the U.S.

	Botany Bay Island, SC (n = 10)						W FL[a]		E FL[a]		NC[a]	
Compound	% >RL	Median	Mean	SE	Min	Max	Median	SE	Median	SE	Median	SE
PCB 99	100	46.9	61.5	26.6	4.48	285	0.888[b]	0.679	4.58[b]	10.5	78.2	28.3
PCB 105	100	19.1	21.1	6.6	2.35	61.5	0.528[b]	0.425	3.21	10.0	43.2	24.0
PCB 118	100	78.8	116	55	9.91	592	1.59[b]	1.32	10.7[b]	20.6	200	56
PCB 128	100	15.4	19.8	7.4	2.34	78.4	0.432[b]	0.292	2.89	4.47	40.9	15.9
PCB 138	100	87.2	87.5	27.1	12.0	290	0.445[b]	1.61	12.0[b]	26.9	165	97
PCB 153 + 132	100	199	222	65	41.1	677	5.26[b]	5.83	49.0	42.9	233	125
PCB 170	100	9.60	13.4	3.9	3.42	35.6	0.343[b]	0.315	3.79[b]	2.61	12.2	8.2
PCB 180 + 193	100	20.8	31.0	9.4	8.97	87.8	0.914[b]	1.07	7.78	15.46	36.2	22.4
PCB 187	100	38.0	43.4	13.2	6.93	131	0.600[b]	0.454	3.60[b]	6.84	53.5	30.9
PCB 199	100	4.51	24.6	19.2	1.77	197	0.470[b]	0.165	1.52[b]	1.32	7.1	4.7
PCB 206	100	2.14	19.3	15.9	1.18	163	0.421[b]	0.055	0.755[b]	0.9	2.14	1.38
$\sum_{13}$PCBs	100	527	659	216	107	2220	11.0[b]	12.3	109[b]	131	876	405
Ratio of PCBs 206:(153 + 132)	100	0.0178	0.0741	0.0381	0.00607	0.393	0.0184	0.00885	0.0177	0.00355	0.0116	0.00292
4,4′-DDE	100	125	325	185	29.4	1970	12.4[b]	7.0	55.0	55.7	824	250
$\sum_{6}$DDTs	100	125	325	185	30.8	1970	13.9[b]	7.1	55.0	55.9	829	251
oxychlordane	100	15.8	37.3	16.8	4.70	176	2.67[b]	5.52	19.9	13.0	105	57
trans-chlordane	100	4.88	11.5	5.6	0.938	59.7	<0.591[b]	NA	0.183[b]	0.094	<0.669[b]	NA
cis-chlordane	60	0.590	0.801	0.183	<0.477	2.37	1.09	NA	0.444	0.019	<0.648	NA
trans-nonachlor	100	35.2	44.2	18.9	6.39	207	1.88[b]	2.68	15.7	15.1	145	68
cis-nonachlor	100	1.25	1.48	0.30	0.376	2.95	0.126[b]	0.117	0.559	0.300	4.16	1.61
$\sum_{5}$chlordanes	100	58.4	94.9	41.2	13.0	448	4.06[b]	8.23	49.7	27.4	299	124
mirex	100	2.16	6.11	2.85	1.35	30.2	0.174[b]	0.529	1.84	3.78	9.56	3.00
PBDE 100	60	1.02	1.61	0.27	<0.599	3.13	0.243[b]	0.027	0.261	0.143	2.73	1.61
PBDE 153	40	0.179	0.988	0.457	<0.654	4.76	<0.156	NA	0.111	0.094	0.685	0.146
PBDE 154	30	0.459	1.27	0.52	<1.01	4.56	<0.0504	NA	0.031	0.160	3.09	1.34
$\sum_{5}$PBDEs	60	1.02	3.36	1.31	0.599	12.1	0.664	0.202	1.44	0.55	7.80	4.75
α-HBCD	10	<0.210	<0.213	NA	<0.163	0.277	NA	NA	NA	NA	NA	NA
Total extractable organics (%)	100	6.10	6.15	0.30	4.74	7.66	8.42	0.82	7.40	0.54	7.41	0.33

[a] Values from Alava et al. [2]; some totals were recalculated from original data for the best comparison to the present study.
[b] Indicates a significant difference from South Carolina eggs ($p < 0.017$).
SC = South Carolina; NC = North Carolina; E FL = eastern Florida; W FL = western Florida; SE = standard error; PCB = polychlorinated biphenyl; PBDE = polybrominated diphenylether; DDE = dichlorodiphenyldichloroethylene; DDT = dichlorodiphenyltrichloroethane; HBCD = hexabromocyclododecane; NA = not available.

classes was 10%), and there was no gradient from the top to the bottom of the nests, suggesting that all eggs in a single clutch have similar POP concentrations. This confirms the low relative standard deviations previously measured in sea turtle nests [12,13] and suggests that egg order in the oviduct does not affect POP concentrations as was previously suspected in snapping turtles. The tendency for snapping turtle eggs found at the bottom of a nest to have slightly higher POP concentrations than eggs higher in the same nest could be an artifact of low sample size in the previous study (four nests were analyzed) [19] or a difference in yolk deposition physiology between freshwater and sea turtles. Regardless, this finding suggests that any egg collected for the BEMAST specimen banking project,

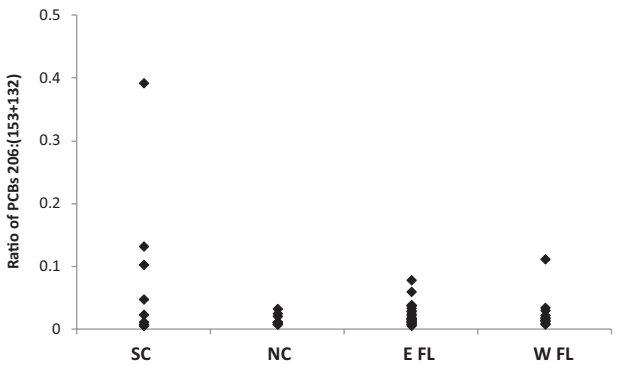

Fig. 2. Concentration ratios of polychlorinated biphenyl congeners (PCB 206 to PCB 153 + 132), as an indicator of exposure to Aroclor 1268, in loggerhead turtle egg samples from four regions along the southeastern U.S. coast. Data points are individual nests. SC = South Carolina (data are from the current study); NC = North Carolina; E FL = eastern Florida; W FL = western Florida (data are taken from Alava et al [2]).

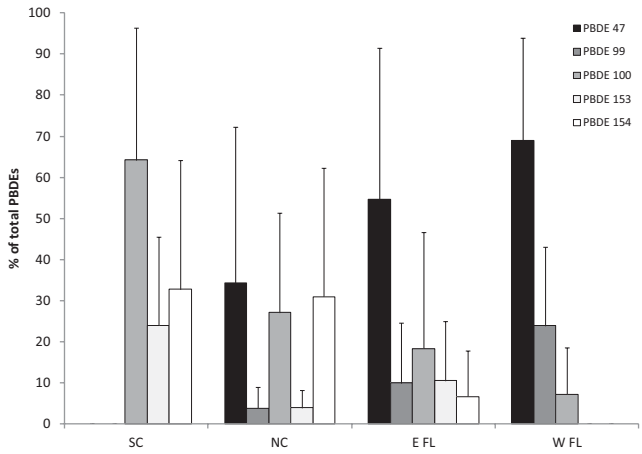

Fig. 3. Polybrominated diphenylether (PBDE) congener profiles in egg samples from loggerhead nests from four regions along the southeastern U.S. coast. Data are means and one standard deviation across nests. SC = South Carolina (data are from the present study); NC = North Carolina; E FL = eastern Florida; W FL = western Florida (data are taken from Alava et al [2]).

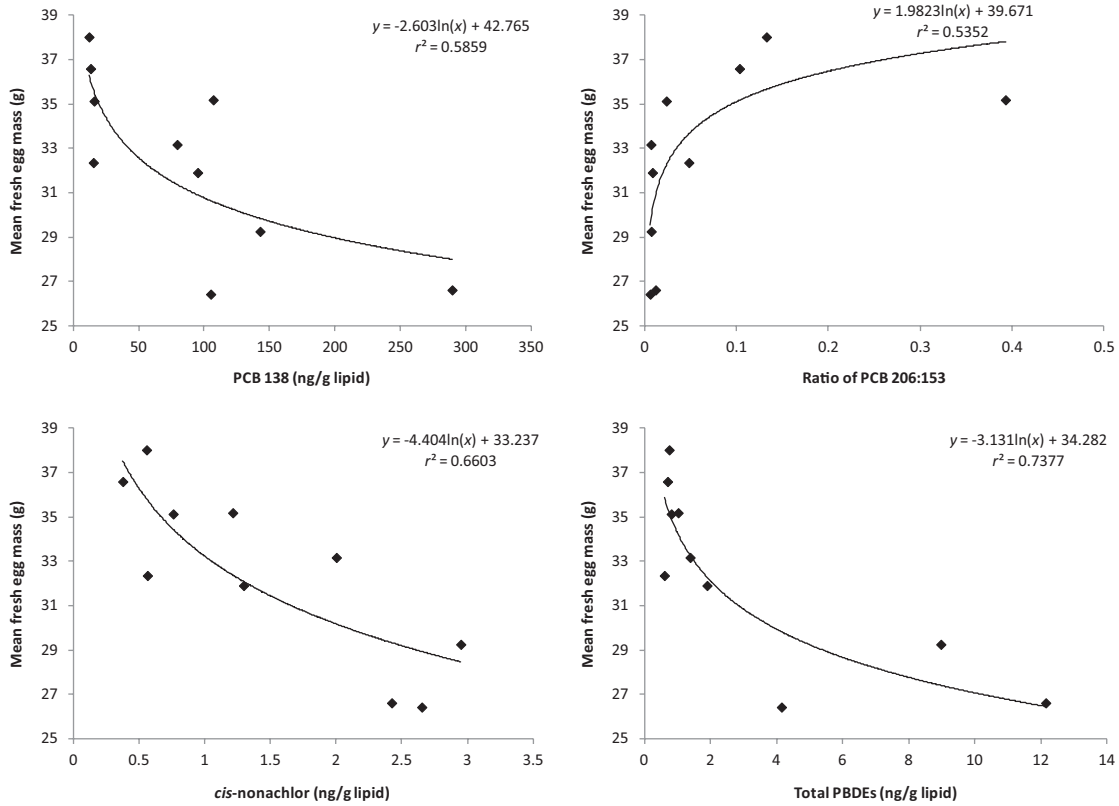

Fig. 4. Logarithmic relationships between persistent organic pollutant concentrations or ratios in 10 loggerhead turtle nests and mean mass of fresh egg contents. PCB = polychlorinated biphenyl; PBDE = polybrominated diphenylether.

regardless of its location within the nest, should represent the POP concentration of that nest.

Collection of unhatched eggs during a nest inventory is a nonlethal sampling technique that is preferred by federal and state conservation managers because taking a fresh egg sacrifices a potential future hatchling. Most sea turtle nests have hatch success rates that allow for the collection of many unhatched eggs [14], and the mean hatch success of 73% from these 10 nests agrees with that previous conclusion. Unhatched eggs had the same POP concentrations as fresh eggs, indicating that decomposition and exposure to the physical conditions of the beach environment do not influence compound concentrations. It validates the use of unhatched eggs in previously published monitoring studies [2,11,13,18] and confirms that fresh eggs of endangered or threatened sea turtle species do not need to be sacrificed for monitoring POPs.

Geographical comparisons of POP concentrations can now be made from loggerhead turtles nesting in North Carolina, South Carolina, and eastern and western Florida (Table 2). Concentrations in South Carolina nests were most similar to those in North Carolina nests. Both South Carolina and North Carolina nests were higher than Florida nests, with eastern Florida having higher concentrations than western Florida [2]. Alava et al. [2] reviewed the available tracking data of nesting loggerhead turtles (see Fig. 3 in that citation) and concluded that spatial differences in POP levels were due to different adult foraging areas. As new satellite tracks become available [21,22,32], the differences in preferred adult foraging areas are becoming more apparent. Generally, postnesting loggerhead turtles from the genetically distinct Northern Recovery Unit (those nesting in Virginia, North Carolina, South Carolina, and Georgia [15]) prefer to forage in Continental Shelf waters from Georgia to New Jersey, with fewer foraging off eastern Florida and as far south as northern Cuba [21–23] (D. Griffin et al., South Carolina Department of Natural Resources, Charleston, SC, USA, unpublished data). Nesting beach selection (Georgia, North Carolina, or South Carolina) does not appear to be influenced by the foraging strategy (determined by location of the adult foraging area) [22] (D. Griffin, South Carolina Department of Natural Resources, Charleston, SC, USA, personal communication), which supports the similarity of POP concentrations in eggs from nests laid in North Carolina and South Carolina. The majority of western Florida nesting females forage along the western Florida coast, with smaller percentages choosing the Yucatan Peninsula, northern Cuba to the Bahamas, or the Louisiana to Florida panhandle area [32,33]. It is clear, by combining the tracking and contaminant data, that regions in the Gulf of Mexico are less contaminated by POPs than regions used by the Northern Recovery Unit in the Mid-Atlantic and South Atlantic Bights. Eastern Florida nesting loggerheads choose a wide range of adult foraging areas that overlap with all areas used by Northern Recovery Unit and western Florida nesting loggerheads [34,35]. This may explain why their POP concentrations are intermediate. North–south latitudinal gradients in POP levels have been seen before in loggerhead eggs [2], adult male loggerhead plasma [29], and juvenile loggerhead plasma [36]. These baseline POP concentrations in a new location (South Carolina) further support the idea that loggerhead turtles can be good indicators of region-scale marine contamination. Even though they are highly migratory, their exposure to a particular concentration and pattern of contaminants is constrained because of their strong site fidelity [22]. Taken together, these studies indicate that loggerhead turtles foraging in more northern regions of the

northwest Atlantic Ocean are exposed to higher POP concentrations and, thus, should be the focus of future health and toxicity studies.

Aside from the spatial trends addressed above, a temporal comparison can be made between these PCB concentrations and those measured in loggerhead eggs from South Carolina two decades ago. In 1993, Cobb and Wood [17] collected unhatched loggerhead eggs from four nests on Cape Island, South Carolina, USA, and measured a mean of 2,556 ng/g lipid with a standard error of 1,202 ng/g lipid for $\sum_{66}$PCBs in the egg contents. Based on North Carolina loggerhead egg data in Alava et al. [2], the 13 PCB congeners summed in the current study ($\sum_{13}$PCBs) contribute 85% to the total PCB concentrations ($\sum_{49}$PCBs), which would translate to a mean of approximately 758 ng/g lipid in these more recent South Carolina samples. This comparison suggests that PCB concentrations have declined approximately 30% at maternal adult foraging areas over 17 years, which may not be surprising since PCBs were banned from use in the United States in the late 1970s. Arguably, though, this finding was derived from a simple two–time point comparison, and the findings are complex from the few other temporal trends from this region. Concentrations of PCBs significantly declined in mollusks from 1965 to 1993 [37]; however, increases have been documented in coastal Florida marine mammals and sharks over a time period (1990s–2000s) similar to the sea turtle comparison [38]. Temporal trends for the other POPs cannot be assessed due to a lack of past baseline data in sea turtle eggs.

At least one South Carolina nest had an elevated ratio of PCB 206 to PCB 153 + 132, suggesting that it may be exposed to the unusual mixture of highly chlorinated PCBs known to contaminate a Superfund site in Brunswick, Georgia, USA. Botany Bay Plantation is 277 km (driving distance) from Brunswick, Georgia, which is closer than any other nesting beach tested yet for POPs (Cape Lookout, NC, USA, is 740 km away; Melbourne Beach, FL, USA, the northernmost eastern Florida beach monitored, is 404 km away). The proximity of the nesting beach does not automatically increase the chance of exposure to the Aroclor 1268 mixture from this Superfund site because the majority of the turtles travel far distances to reach their preferred adult foraging areas, commonly anywhere from Cuba to New Jersey for turtles from the Northern Recovery Unit [21,22]. Only one of the 15 satellite-tracked nesting females from South Carolina chose an adult foraging area off the coast of Georgia (D. Griffin, South Carolina Department of Natural Resources, Charleston, SC, USA, personal communication). More research is needed to understand how far the contamination of Aroclor 1268 has spread from the Brunswick Superfund site and to determine the exposure of turtles known to forage in coastal areas near this site.

Another interesting contamination pattern seen in sea turtles is an unusual profile of PBDEs observed in various species sampled from 30 to 40°N latitude along the east coast of the United States [2,29] (B. Carlson, 2006, Master's thesis, College of Charleston, Charleston, SC, USA). Most wildlife accumulate a pattern with concentrations of PBDE congeners in this order: $47 > 99 \approx 100 > 153 \approx 154$ [31]. This typical pattern was seen in loggerhead turtle eggs from eastern and western Florida but not in eggs from North Carolina [2] or South Carolina. In North Carolina eggs, PBDEs 100 and 154 made up a larger percentage of the total PBDEs than expected. In South Carolina eggs, the pattern was even more unusual, with PBDEs 100 and 154 predominating and no PBDEs 47 or 99 detected. This finding supports several previous reptile studies showing this unusual pattern [2,29,39,40] (B. Carlson, 2006, Master's thesis, College of Charleston, Charleston, SC, USA), and furthermore, it suggests that this unusual PBDE pattern may originate from regions within adult foraging areas of the Northern Recovery Unit from Cuba to New Jersey, rather than the Caribbean or Gulf of Mexico. The use of individual PBDE congeners is not common, and these congeners (PBDEs 100 and 154) are known to come from the penta-BDE technical mixture, which contains more PBDEs 47 and 99; so the predominance of these congeners remains unexplained.

The POP concentrations measured in the present study correlated with marginal statistical significance ($p < 0.05$) to only one reproductive success variable, reduced fresh egg mass. It should be mentioned that this correlation cannot elucidate whether female loggerhead turtles that had higher POP concentrations were unable to produce larger eggs or if females with greater egg masses diluted their POP concentrations. However, evidence is growing in several recent bird studies that POPs can cause a reduction in egg size [41–44]. This is important because, at least in birds, egg size is an important predictor of hatchling and juvenile fitness [45]. Therefore, contaminant exposure could plausibly lead to smaller eggs and less fit hatchlings, thereby reducing the survival and population growth of this threatened species. The lack of correlations with other reproductive variables could indicate that (1) these contaminants are not influencing the reproductive success of loggerhead turtles, (2) a sample size of eight to ten is insufficient to detect significant relationships, or (3) these reproductive success variables are not the most sensitive measurements of adverse effects of contaminants. Future studies should consider a larger sample of nests and include additional, more sensitive variables that have been shown previously to relate to POP concentrations, such as hatchling body condition (negatively correlated with higher POP concentrations in green sea turtle eggs [12]), neurobehavioral tests (righting response times were slower in diamondback terrapin [*Malachlemys terrapin*] hatchlings from nests with higher PBDE 47 concentrations [46]), sex ratios (skewed in red-eared sliders [*Trachemys scripta*] exposed to PCBs in the laboratory [47]), and hatchling growth rates (slower in diamondback terrapins exposed to PCB 126 in the laboratory [48]).

As another way to preliminarily assess toxicity risk, POP concentrations measured in the current study were compared to previously published levels in wild reptile eggs from highly contaminated sites where endocrine disruption has been observed, leading to altered sexually dimorphic characteristics or reproductive organs [26,27,49]. Snapping turtle eggs from a highly PCB-contaminated site in Lake Ontario (Hamilton Harbor) showing signs of endocrine disruption had a mean $\sum_{59}$PCB mass fraction (or concentration) of 43,157 ng/g lipid [26], which is 57 times higher than that in the South Carolina loggerhead turtle eggs. This difference affords a margin of safety of less than 100, which is not considered protective against risk by regulatory agencies [50] and suggests that loggerhead turtles may be at risk of reproductive or developmental toxic effects from PCB exposure. American alligator eggs from Lake Apopka, Florida, USA, where a pesticide spill was suspected to cause a significant population decline and long-lasting endocrine-disrupting effects [27], had 4,4′-dichlorodiphenyldichloroethylene mass fractions of 5,800 ng/g wet mass (or 58,000 ng/g lipid using 10% lipid as an estimate) [49]. This concentration is 178 times higher than those of the 10 loggerhead turtle nests in the present study. These comparisons suggest that the average loggerhead hatchlings from South

Carolina beaches have POP concentrations lower than those of wild reptiles inhabiting grossly contaminated areas where toxic effects have been observed. However, there is still potential risk because we do not know the sensitivity of sea turtles to these toxic compounds and the eggs have a margin of safety of less than 100 for toxic effects of PCBs.

CONCLUSIONS

The present study confirms that the NIST sea turtle specimen bank (BEMAST) or any contaminant-monitoring program can collect unhatched eggs with the knowledge that any egg represents the POP burden of that nest regardless of its nest depth and that sacrificing a fresh egg is not necessary. The Northern Recovery Unit of the northwest Atlantic distinct population segment [15] loggerhead sea turtle is a small, genetically distinct population nesting from Georgia to Virginia. It is faced with the highest POP exposure compared to other tested populations (Table 2). Its foraging areas encompass offshore waters from New Jersey to Cuba, including shelf waters of Georgia which could be a source of PCB exposure from the Brunswick, Georgia, USA, Superfund site [21–23]; and it demonstrates exposure to an unexplained, unusual PBDE pattern (Fig. 3). Future studies should investigate the spatial gradient of PCB contamination in prey extending from Brunswick, Georgia, and analyze samples from sea turtles that are known to forage in this locality. In addition, investigations into the reason for the unusual PBDE pattern seemingly localized to turtles from the Northern Recovery Unit should be conducted as well as toxicity studies that address the sensitivity of sea turtle hatchlings to these POP exposures.

SUPPLEMENTAL DATA

Table S1–S2. (70 KB DOC).

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Disclaimer—Certain commercial equipment, instruments, or materials are identified in this article to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

REFERENCES

1. de Solla SR, Fernie KJ, Letcher RJ, Chu SG, Drouillard KG, Shahmiri S. 2007. Snapping turtles (*Chelydra serpentina*) as bioindicators in Canadian areas of concern in the Great Lakes basin. 1. Polybrominated diphenyl ethers, polychlorinated biphenyls, and organochlorine pesticides in eggs. *Environ Sci Technol* 41:7252–7259.
2. Alava JJ, Keller JM, Wyneken J, Crowder L, Scott G, Kucklick JR. 2011. Geographical variation of persistent organic pollutants in eggs of threatened loggerhead sea turtles (*Caretta Caretta*) from southeastern United States. *Environ Toxicol Chem* 30:1677–1688.
3. Vander Pol SS, Becker PR, Kucklick JR, Pugh RS, Roseneau DG, Simac KS. 2004. Persistent organic pollutants in Alaskan murre (*Uria* spp.) eggs: Geographical, species, and temporal comparisons. *Environ Sci Technol* 38:1305–1312.
4. Miller JD, Limpus CJ, Godfrey MH. 2003. Nest site selection, oviposition, eggs, development, hatching, and emergence of loggerhead turtles. In Bolten AB, Witherington BE, eds, *Loggerhead Sea Turtles*. Smithsonian Books, Washington, DC, USA, pp 125–143.
5. Schroeder BA, Foley AM, Bagley DA. 2003. Nesting patterns, reproductive migrations, and adult foraging areas of loggerhead turtles. In Bolten AB, Witherington BE, eds, *Loggerhead Sea Turtles*. Smithsonian Books, Washington, DC, USA, pp 114–124.
6. Sellström U, Bignert A, Kierkegaard A, Haggberg L, de Wit CA, Olsson M, Jansson B. 2003. Temporal trend studies on tetra- and pentabrominated diphenyl ethers and hexabromocyclododecane in guillemot egg from the Baltic Sea. *Environ Sci Technol* 37:5496–5501.
7. Ward J, Mohapatra S, Mitchell A. 2008. An overview of policies for managing polybrominated diphenyl ethers (PBDEs) in the Great Lakes basin. *Environ Int* 34:1148–1156.
8. Klein R, Bartel-Steinbach M, Koschorreck J, Paulus M, Tarricone K, Teubner D, Wagner G, Weimann T, Veith M. 2012. Standardization of egg collection from aquatic birds for biomonitoring—A critical review. *Environ Sci Technol* 46:5273–5284.
9. Pugh RS, Becker PR, Porter BJ, Ellisor MB, Moors AJ, Wise SA. 2008. Design and applications of the National Institute of Standards and Technology's (NIST's) environmental specimen banking programs. *Cell Preserv Technol* 6:59–72.
10. Keller JM. 2013. Exposure to and effects of persistent organic pollutants. In Wyneken J, Lohmann KJ, Musick JA, eds, *Biology of Sea Turtles*, Vol 3. CRC/Taylor & Francis, Boca Raton, FL, USA.
11. Stewart KR, Keller JM, Templeton R, Kucklick JR, Johnson C. 2011. Monitoring persistent organic pollutants in leatherback turtles (*Dermochelys coriacea*) confirms maternal transfer. *Mar Pollut Bull* 62:1396–1409.
12. van de Merwe JP, Hodge M, Whittier JM, Ibrahim K, Lee SY. 2010. Persistent organic pollutants in the green sea turtle *Chelonia mydas*: Nesting population variation, maternal transfer, and effects on development. *Mar Ecol Prog Ser* 403:269–278.
13. Alava JJ, Keller JM, Kucklick JR, Wyneken J, Crowder L, Scott GI. 2006. Loggerhead sea turtle (*Caretta caretta*) egg yolk concentrations of persistent organic pollutants and lipid increase during the last stage of embryonic development. *Sci Total Environ* 367:170–181.
14. Miller JD. 1996. Reproduction in sea turtles. In Lutz PL, Musick JA, eds, *The Biology of Sea Turtles*. CRC, Boca Raton, FL, USA, pp 51–81.
15. National Marine Fisheries Service and U.S. Fish and Wildlife Service. 2008. Recovery plan for the northwest Atlantic population of the loggerhead sea turtle (*Caretta caretta*), 2nd Rev. National Marine Fisheries Service, Silver Spring, MD.
16. Witherington B, Kubilis P, Brost B, Meylan A. 2009. Decreasing annual nest counts in a globally important loggerhead sea turtle population. *Ecol Appl* 19:30–54.
17. Cobb GP, Wood PD. 1997. PCB concentrations in eggs and chorioallantoic membranes of loggerhead sea turtles (*Caretta caretta*) from the Cape Romain National Wildlife Refuge. *Chemosphere* 34: 539–549.
18. Mckenzie C, Godley BJ, Furness RW, Wells DE. 1999. Concentrations and patterns of organochlorine contaminants in marine turtles from Mediterranean and Atlantic waters. *Mar Environ Res* 47:117–135.
19. Bishop C, Lean D, Brooks R. 1995. Chlorinated hydrocarbons in early-life stages of the common snapping turtle (*Chelydra serpentina serpentina*) from a coastal wetland on Lake Ontario, Canada. *Environ Toxicol Chem* 14:421–426.
20. Hopkins-Murphy SR, Owens DW, Murphy TM. 2003. Ecology of immature loggerheads on foraging grounds and adults in internesting habitat in the eastern United States. In Bolten AB, Witherington BE, eds, *Loggerhead Sea Turtles*. Smithsonian Institute, Washington, DC, USA, pp 79–92.
21. Hawkes LA, Broderick AC, Coyne MS, Godfrey MH, Godley BJ. 2007. Only some like it hot—Quantifying the environmental niche of the loggerhead sea turtle. *Divers Distrib* 13:447–457.
22. Hawkes LA, Witt MJ, Broderick AC, Coker JW, Coyne MS, Dodd M, Frick MG, Godfrey MH, Griffin DB, Murphy SR, Murphy TM, Williams KL, Godley BJ. 2011. Home on the range: Spatial ecology of loggerhead turtles in Atlantic waters of the USA. *Divers Distrib* 17:624–640.
23. Plotkin PT, Spotila JR. 2002. Post-nesting migrations of loggerhead turtles *Caretta caretta* from Georgia, USA: Conservation implications for a genetically distinct subpopulation. *Oryx* 36:396–399.
24. Balmer BC, Schwacke LH, Wells RS, George RC, Hoguet J, Kucklick JR, Lane SM, Martinez A, McLellan WA, Rosel PE, Rowles TK, Sparks K, Speakman T, Zolman ES, Pabst DA. 2011. Relationship between persistent organic pollutants (POPs) and ranging patterns in common bottlenose dolphins (*Tursiops truncatus*) from coastal Georgia, USA. *Sci Total Environ* 409:2094–2101.

25. Maruya KA, Lee RF. 1998. Aroclor 1268 and toxaphene in fish from a southeastern U.S. estuary. *Environ Sci Technol* 32:1069–1075.
26. de Solla SR, Bishop CA, Brooks RJ. 2002. Sexually dimorphic morphology of hatchling snapping turtles (*Chelydra serpentina*) from contaminated and reference sites in the Great Lakes and St. Lawrence River basin, North America. *Environ Toxicol Chem* 21:922–929.
27. Guillette LJ Jr, Pickford DB, Crain DA, Rooney AA, Percival HF. 1996. Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. *Gen Comp Endocr* 101:32–42.
28. Miller JD. 1999. Determining clutch size and hatching success. In Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M, eds, *Research and Management Techniques for the Conservation of Sea Turtles*. IUCN/SSC Marine Turtle Specialist Group Publication 4, Washington, DC, USA, pp 124–129.
29. Ragland JM, Arendt MD, Kucklick JR, Keller JM. 2011. Persistent organic pollutants in blood plasma of satellite-tracked adult male loggerhead sea turtles (*Caretta caretta*). *Environ Toxicol Chem* 30:1549–1556.
30. Helsel DR. 2005. *Nondetects and Data Analysis: Statistics for Censored Environmental Data*. John Wiley & Sons, Hoboken, NJ, USA.
31. Hites RA. 2004. Polybrominated diphenyl ethers in the environment and in people: A meta-analysis of concentrations. *Environ Sci Technol* 38:945–956.
32. Hart KM, Lamont MM, Fujisaki I, Tucker AD, Carthy RR. 2012. Common coastal foraging areas for loggerheads in the Gulf of Mexico: Opportunities for marine conservation. *Biol Conserv* 145:185–194.
33. Girard C, Tucker AD, Calmettes B. 2009. Post-nesting migrations of loggerhead sea turtles in the Gulf of Mexico: Dispersal in highly dynamic conditions. *Mar Biol* 156:1827–1839.
34. Turtle Expert Working Group. 2009. An assessment of the loggerhead turtle population in the western north Atlantic Ocean. National Oceanic and Atmospheric Adminiatration Technical Memorandum NMFS-SEFSC-575, Miami, FL, USA, pp. 131.
35. Meylan AB, Bjorndal KA. 1983. Sea turtles nesting at Melbourne Beach, Florida, II. Post-nesting movements of *Caretta caretta*. *Biol Conserv* 26:79–90.
36. O'Connell SG, Arendt M, Segars A, Kimmel T, Braun-McNeill J, Avens L, Schroeder B, Ngai L, Kucklick JR, Keller JM. 2010. Temporal and spatial trends of perfluorinated compounds in juvenile loggerhead sea turtles (*Caretta caretta*) along the east coast of the United States. *Environ Sci Technol* 44:5202–5209.
37. Lauenstein GG, Daskalakis KD. 1998. U.S. long-term coastal contaminant temporal trends determined from mollusk monitoring programs, 1965–1993. *Mar Pollut Bull* 37:6–13.
38. Johnson-Restrepo B, Kannan K, Addink R, Adams DH. 2005. Polybrominated diphenyl ethers and polychlorinated biphenyls in a marine foodweb of coastal Florida. *Environ Sci Technol* 39:8243–8250.
39. Basile ER, Avery HW, Keller JM, Bien WF, Spotila JR. 2011. Diamondback terrapins as indicator species of persistent organic pollutants: Using Barnegat Bay, New Jersey as a case study. *Chemosphere* 82:137–144.
40. Moss S, Keller JM, Richards S, Wilson TP. 2009. Concentrations of persistent organic pollutants in plasma from two species of turtle from the Tennessee River Gorge. *Chemosphere* 76:194–204.
41. Fernie KJ, Marteinson SC, Bird DM, Ritchie IJ, Letcher RJ. 2011. Reproductive changes in American kestrels (*Falco sparverius*) in relation to exposure to technical hexabromocyclododecane flame retardant. *Environ Toxicol Chem* 30:2570–2575.
42. Fernie KJ, Shutt JL, Letcher RJ, Ritchie IJ, Bird DM. 2009. Environmentally relevant concentrations of DE-71 and HBCD alter eggshell thickness and reproductive success of American kestrels. *Environ Sci Technol* 43:2124–2130.
43. Marteinson SC, Letcher RJ, Graham L, Kimmins S, Tomy G, Palace VP, Ritchie IJ, Gauthier LT, Bird DM, Fernie KJ. 2012. The flame retardant beta-1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane: Fate, fertility, and reproductive success in American kestrels (*Falco sparverius*). *Environ Sci Technol* 46:8440–8447.
44. Verboven N, Verreault J, Letcher RJ, Gabrielsen GW, Evans NP. 2009. Differential investment in eggs by Arctic-breeding glaucous gulls (*Larus hyperboreus*) exposed to persistent organic pollutants. *Auk* 126:123–133.
45. Krist M. 2011. Egg size and offspring quality: A meta-analysis in birds. *Biol Rev* 86:692–716.
46. Basile ER. 2010. Persistent organic pollutants in diamondback terrapin (*Malaclemys terrapin*) tissues and eggs, and sediments in Barnegat Bay, New Jersey. PhD dissertation. Drexel University, Philadelphia, PA, USA.
47. Bergeron JM, Crews D, McLachlan JA. 1994. PCBs as environmental estrogens: Turtle sex determination as a biomarker of environmental contamination. *Environ Health Perspect* 102:780–781.
48. Holliday DK, Elskus AA, Roosenburg WM. 2009. Impacts of multiple stressors on growth and metabolic rate of Malaclemys terrapin. *Environ Toxicol Chem* 28:338–345.
49. Heinz GH, Percival HF, Jennings ML. 1991. Contaminants in American alligator eggs from Lake Apopka, Lake Griffin, and Lake Okeechobee, Florida. *Environ Monit Assess* 16:277–285.
50. Faustman EM, Omenn GS. 1996. Risk assessment. In Klaassen CD, ed, *Casarett and Doull's Toxicology*, 5th ed. McGraw-Hill, New York, NY, USA, pp 75–88.