Running Head: Spatial differences in loggerhead egg POPs along Southeast US

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Geographical variation of Persistent Organic Pollutants in eggs of threatened loggerhead sea turtles (*Caretta caretta*) from Southeastern USA

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Abstract

Persistent organic pollutants (POPs) are a recognized man-made threat to sea turtle populations, but the uncertainty surrounding exposure and sensitivity of sea turtles to contaminants is great and makes decision making difficult for conservation managers. To provide baseline concentrations and spatial comparisons, we measured a large suite of POPs in loggerhead sea turtle (*Caretta caretta*) egg yolk samples from 44 nests laid in three distinct locations: North Carolina (NC), eastern Florida (E FL), and western Florida (W FL). POPs included polychlorinated biphenyls (PCBs), organochlorine pesticides such as DDTs, chlordanes, mirex, dieldin, HCHs, HCB, and toxaphenes, as well as polybrominated diphenyl ethers (PBDEs). POP concentrations were lowest in W FL, intermediate in E FL, and highest in NC. This increasing gradient along the southeast coast around the FL peninsula to NC was explained partly by the foraging site selection of the nesting females. 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. 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 atypical PBDE pattern was seen in the NC samples with nearly equal contributions of PBDEs 47, 100 and 154. A future study will assess correlations between these POP concentrations and measures of hatching success and hatchling fitness.

Keywords: reptile, egg, lipid, contaminant, organohalogen

Introduction

Loggerhead sea turtles (*Caretta caretta*) are listed as threatened on the U.S. Endangered Species List, but because of declining nesting trends certain subpopulations, including loggerheads inhabiting the Northwest Atlantic Ocean, have recently been considered for the more protected status of "endangered" (Conant, 2009). Four recovery units have been identified for the Northwest Atlantic loggerhead in the U.S.: the Northern nesting subpopulation, ranging from Virginia to southern Georgia, Peninsular Florida subpopulation, Northern Gulf of Mexico subpopulation, and Dry Tortugas subpopulation (NMFS and USFWS, 2008). The first two subpopulations were sampled in the current study and have been declining by ≈1.6% per year since the 1980s. The list of threats that this species faces is long, ranging from nesting beach habitat destruction, fisheries by-catch, vessel strikes, poaching, diseases, predation, marine debris, to chemical pollutants.

While environmental contaminants are a recognized threat, the uncertainty of the magnitude of the risk they pose is great (NMFS and FWS, 2008) because little to no data exist on the effects of chemicals on sea turtles. Moreover, simple baseline exposure data do not exist for contaminant concentrations in certain subpopulations. For example, the eastern coast of Florida is possibly the largest rookery of loggerheads in the world, rivaled only by Masirah in Oman (NMFS and FWS, 2008), 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 (OCPs) (Alava et al., 2006). Prior to that study, loggerhead eggs from this region had not been collected for POP measurements since the 1970s (Clark and Krynitski, 1985). Since the 1990s, loggerhead eggs from the U.S. have been analyzed for POPs from only South Carolina (Cobb and Wood, 1997), the Florida panhandle (Alam and Brim, 2000), and

eastern Florida (Alava et al., 2006). These three studies took advantage of non-lethal sampling using unhatched eggs after live hatchlings have emerged from the nest. Even so, compiling data from these studies cannot provide a good spatial comparison of POP exposure among the genetically-distinct subpopulations or regions because of temporal differences in sampling (1970s to 2002), focus on different suites of compounds, and analytical method differences (e.g. Alam and Brim (2000) reported on dry-mass whereas all other studies used wet-mass).

In warm months, loggerhead turtles lay three or so clutches of typically over 100 eggs each on nesting beaches (Schroeder et al., 2003; Miller et al., 2003). In the average three years between nesting seasons, many migrate hundreds of kilometers to foraging grounds, often to the same area each remigration, where nutritive resources are used to deposit yolk into follicles for the next season (Schroeder et al., 2003; Miller et al., 2003). During this time, adult females accumulate POPs from their prey and deposit them along with lipids into the follicles. Indeed, maternal transfer of POPs into eggs has been documented in many reptiles (e.g. Kelly et al., 2008) as well as green sea turtles (*Chelonia mydas*) and leatherback sea turtles (*Dermochelys coriacea*) (van de Merwe et al., 2010; Guirlet et al., 2010; Stewart et al., in review). This means that POP concentrations in eggs represent the contamination at the foraging grounds of that adult female and that if the females nesting on the same beach forage in varied locations then POP concentrations could be quite variable among nests on a single nesting beach. Alternatively, if females from one nesting beach forage in similar locations then their egg POP concentrations could be a good indicator of the contamination in their foraging region.

Presence of POPs in egg yolk represents a risk to the developing embryo. Van de Merwe et al. (2010) showed that POPs transfer from green sea turtle eggs into embryos and that higher egg POP concentrations correlated with a lower mass:length ratio of the hatchlings. Turtles with

lower body condition 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 other reptile species (e.g., Eisenreich et al. 2009; Holliday et al., 2009, Bergeron et al., 1994; Guillette *et al.,* 1999; Rauschenberger et al., 2007; Willingham, 2001; Bishop *et al.,* 1998). Therefore, it is important to know the exposure level of a species to these compounds as well as the spatial structure at the subpopulation or regional level in order to make informed management decisions for their population recovery.

Our objectives were to provide baseline concentrations of a large suite of POPs in loggerhead nests collected in distinct and distant 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 similar locations to foraging grounds. The list of POPs greatly expands on previous research and includes legacy compounds, such as PCBs, DDTs, chlordanes, toxaphenes, mirex, dieldrin, hexachlorocyclohexanes (HCHs), and hexachlorobenzene (HCB), as well as brominated flame retardants, the polybrominated diphenyl ethers (PBDEs).

Material 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 Southeastern U.S. in 2002 (Wyneken et al., 2007). Eggs that failed to hatch were collected during nest inventories into plastic bags from a total 44 nests at three regional locations (Table 1; Figure 1). Nests from Sarasota County, FL (n = 11) were considered from W FL. Nests from Boca Raton (n = 11), Juno Beach (n = 4), Hutchinson Island (n = 5), and

Melbourne Beach (n =4) were grouped as from E FL. Nests from Cape Lookout, NC (n = 9) were considered from NC. Eggs were rinsed inside a fume hood with deionized water to remove sand, opened, and staged to determine embryonic development. Since egg contents were shared for sex determination (gonads of middle to late stage embryos were separated and stored) and contaminant measurements, we decided to store 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 only embryonic stages of no, early, or middle development were pooled per nest (Table 1). Samples from late stage development embryos were excluded to minimize confounding factors, because loggerhead yolk POP concentrations are known to increase through development especially by this late stage (Alava et al., 2006). 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 no, early, or middle development) (Alava et al., 2006), and this has been shown in eggs from other sea turtle species (van de Merwe et al., 2010). Three nests were previously analyzed as individual yolk samples rather than pools (Alava et al., 2006), the average POP concentrations of the no, early and mid-developmental stages were included in the current study.

Calibration Solutions and Quality Control

Calibration solutions of differing concentrations were prepared gravimetrically in iso-octane by combining National Institute of Standards and Technology (NIST) 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, Andover, MA). A six-point calibration curve, ranging from 0.35 ng to 370 ng of each compound contained in the above solutions, was extracted and processed alongside the samples. A three-point calibration curve consisting of four toxaphene compounds (0.5 ng to 0.03 ng) was also prepared gravimetrically, but not extracted alongside samples, to semi-quantitatively 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 curves prior to extraction and contained 4,4'-DDT-d_8, 4,4'-DDE- d_8, 4,4'-DDD- d_8, endosulfan I- d_4, PCB 103, and PCB 198. NIST SRM 1946 Lake Superior Fish Tissue and a cryohomogenized composite of loggerhead sea turtle egg yolks from nest FLBR13 were analyzed as control materials, and three procedural blanks were also processed with the set of samples.

Extraction and clean-up of yolk samples

Pooled, spatula-homogenized yolk samples (7.0 g) were mixed with sodium sulfate and extracted using pressurized fluid extraction as described previously (Alava et al., 2006). Water was removed from extracts with sodium sulfate, and they were reduced to 10 mL in volume by evaporation using purified nitrogen. Total extractable organic (TEO) content, a proxy for lipid content, was determined gravimetrically from a 10% subsample of the extract that was allowed to dry in a tared aluminum pan. The dry TEO residue was weighed to the nearest 0.00001 g. Extracts were cleaned up with size exclusion chromatography as described in Kucklick et al.

(2002) followed by solid phase extraction with alumina columns and fractionation with silica columns as described in Alava et al. (2006).

Determination of POP Concentrations

Both fractions (F1 and F2) from the silica column were analyzed on a gas chromatograph (GC) with dual micro-electron capture detectors (ECD) (Hewlett Packard 6890, Palo Alto, CA) for PCBs and certain OCPs. Compounds were separated (2 µL injection) using two different 60 m columns (DB-5 and DB-XLB; J&W Scientific, Folsom, CA) and other instrument parameters were similar to Kucklick et al. (2002).

Both fractions of each sample were recombined (during this step we lost FLHI11 and FLME14 samples), and 20 µL were injected three times onto a GC equipped with a mass spectrometer (Agilent 6890N/5973 inert, Palo Alto, CA) using a programmable temperature vaporization inlet and selected ion monitoring to confirm concentrations of certain PCBs and OCPs and to quantify the PBDEs. A 60 m DB-5MS column (J&W Scientific) was used for the first injection with electron impact mode for selected PCBs, DDTs, mirex, and lower-brominated PBDEs. The second injection used the same 60 m column with negative chemical ionization (NCI) for toxaphenes, chlordanes, HCHs, HCB, endosulfans, endrin, and dieldrin. All compounds were quantified using PCB 198 as the internal standard, except the endosulfans utilized endosulfan I-d_4. The third injection used NCI 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 PBDEs, which were not detectable. Inlet and instrument parameters can be found in Moss et al. (2009).

The amount of each compound was calculated using linear regressions of at least a three-point calibration curve and ratios to the internal standard compounds. The reporting limit (RL)

was established as the ng in the lowest detectable calibration solution divided by the average sample mass (7 g), except 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 TEO content. Only detected compounds were summed to calculate totals for a contaminant class. ∑PCB was the sum of 49 PCB congeners. ∑chlordane was the sum of heptachlor epoxide, oxychlordane, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, and *trans*-nonachlor; ∑HCH was the sum of α-HCH, β-HCH, and γ-HCH; ∑endosulfan was the sum of endosulfan I, endosulfan II, and endosulfan sulfate; ∑PBDE was the sum of 13 PBDE congeners; and ∑toxaphene 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, Vienna, Austria) using the "NADA" package, which can handle left-censored datasets or those with values <RL as recommended by Helsel (2005). Mean, standard deviation, and median were estimated with Kaplan-Meier or Regression on Order (ROS) models. The choice between the two was based on sample size and detection frequency as recommended in Helsel (2005). 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, ∑PCB, 4,4'-DDE, ∑DDTs, ∑POPs, and TEO content), JMP 5.1 (SAS Institute Inc., Cary, NC) software was used to perform ANOVAs or Welch ANOVAs followed by Tukey-Kramer HSD multiple comparison tests (α =

0.05). For compounds with <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 non-parametric (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 pair-wise 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 principle component analysis (PCA) was conducted to visualize differences among regions in the pattern of POPs. The percent of ΣPOPs for each of the following classes were used in the PCA: ∑PCB, ∑DDTs, ∑chlordanes, mirex, dieldrin, ∑PBDEs, and ∑toxaphenes. Half the RL was substituted for values <RL only for the PCA, and the percentages were scaled and centered.

Results and Discussion

Quality control

The POP concentrations measured in SRM 1946 and the loggerhead egg control material that were processed alongside this set of samples were previously reported as "Rep 2" in Alava et al. (2006). On average, measured concentrations were 6% lower than certified or reference values in SRM 1946 and 21% different than "Rep 1" of the loggerhead egg control material. These differences met our criteria for data quality.

Site differences in TEO content and POP concentrations

TEO content did not differ among the three regions (Table 1) and was on average 7.92% with a range from 2.60% to 13.1%. This average is somewhat lower than the average TEO

content (12.4%) measured in the yolk samples from three loggerhead nests that had similarly staged embryos (no development to mid development) (Alava et al., 2006). The large variation in TEO content in these samples is likely due to dilution of the lipids in the yolk with variable amounts of watery and proteinaceous albumen. Since POPs are associated with the lipids, it is imperative in this study that the POP concentrations be normalized to lipid (or TEO) content to avoid this dilution artifact.

Lipid-normalized POP concentrations were often significantly higher in NC and E FL compared to W FL (Table 2 and Figure 2). Regional differences were observed in all but one of the predominant PCB congeners, and total PCBs were significantly higher in NC and E FL than in W FL (E FL was marginally significantly higher than W FL; $p=0.023$ for pairwise comparison). Some of the less predominant compounds, such as total HCHs, HCB, 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 higher in concentration in NC than W FL with E FL being intermediate. The predominant chlordanes (*trans*-nonachlor, oxychlordane, and heptachlor epoxide) resulted in total chlordanes being higher in NC than the FL regions. Not surprisingly, 4,4'-DDE was the predominant DDT metabolite in all samples, and its concentrations were higher in NC than W FL with E FL having similar concentrations to the these two regions. 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 portray an increasing gradient in POP concentrations along the southeast coast from WFL around the FL peninsula northward to NC.

The site differences in egg concentrations suggest that the adult females nesting at these sites chose different foraging grounds. Based on a compilation of available and published tracking data, this suggestion is true (Figure 3). Loggerhead turtles nesting in Sarasota County

(our W FL site) were satellite tagged and tracked as they made their way to foraging locations after the nesting season (Girard et al., 2009). All of these turtles stayed within the Gulf of Mexico or near the Bahamas, Cuba, or Dominican Republic. Loggerhead turtles nesting on Melbourne Beach (one of our E FL beaches) have been flipper tagged for decades. Based on tag return data (Meylan and Bjorndal 1983), it is known that these turtles inhabit a wide range of locations after nesting, including the Gulf of Mexico, Bahamas, Cuba, the eastern coast of FL, as well as the coastline from GA to NJ. Since tag return data can be biased, these proportions and destinations were confirmed with publically available, recent satellite tracks from loggerheads nesting in the Archie Carr National Wildlife Refuge from Melbourne Beach to Wabasso Beach (Sea Turtle Conservancy, 2010). Loggerhead turtles nesting in the Northern subpopulation (near our NC site) on Bald Head Island, NC, and Wassaw, GA, were satellite tracked as they migrated to mainly the coastline of GA to NJ (76%) to forage with 24% destined for eastern FL (Hawkes et al. 2007; Plotkin and Spotila 2000). This larger picture of post-nesting migration information demonstrates that loggerheads nesting in NC utilize a very different region for foraging than those nesting in W FL, and E FL turtles are intermediate. Since the tracking and contaminant data align, loggerhead eggs are a good indicator or integration of regional contamination from the foraging grounds of the adult females. These data also show that the Gulf of Mexico, Caribbean areas, and coastal Florida marine waters are less contaminated with these POPs than the coastal waters of GA to NJ.

Site differences in POP concentrations have been noted in loggerhead sea turtles along the US East Coast in three previous studies (Keller et al., 2005; O'Connell et al., 2010; Ragland et al., in review). Keller et al. (2005) observed higher plasma concentrations of perfluorinated contaminants (PFCs) in juvenile loggerheads captured in NC than northern FL. O'Connell et al.

(2010) expanded this spatial assessment of PFCs and found that plasma concentrations of the predominant PFC, perfluorooctane sulfonate (PFOS), was higher in juvenile loggerheads captured in MD and NC as compared to Cape Canaveral, FL. Since the PFCs come from different anthropogenic sources and have different environmental transport mechanisms than the POPs measured in this study, a better comparison is to the study by Ragland et al. (in review). They found that adult male loggerhead sea turtles that migrated north and chose foraging habitats along the coastline between SC and NJ had higher concentrations of POPs than males that remained resident at the capture site of Cape Canaveral, FL. Interestingly, these three studies support the conclusion of the current study that sea turtles foraging further north have higher concentrations of POPs.

The reasons for this North-South gradient are unknown but are likely a very complicated combination of factors. O'Connell et al. (2010) showed that PFC concentrations in loggerhead turtles correlated with human abundance within the watershed draining into the respective turtle capture locations. Thus, simply the number of people residing and using chemicals within a watershed affect what is available for sea turtles to accumulate, but this logic cannot help 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 high human population. Thus, other factors must be involved, including atmospheric transport of POPs away from these warmer southern waters towards the north or more sedimentation burying the POPs as they enter the coastal regions.

Site differences in POP patterns

∑PCBs were the dominant group of compounds in all nests, but their contribution to ∑POPs differed among regions (Figure 4A). ∑PCBs represented 33% on average of ∑POPs in

W FL, 49% in E FL, and 63% in NC. ∑DDTs and ∑chlordanes were the next highest class of contaminant measured in all three regions, followed by dieldrin. Mirex, ∑PBDEs, and ∑toxaphenes made up minor contributions to ∑POPs.

The PCA resulted in the first two principle components (PCs) accounting for 57% of the variation in POP patterns and large overlap among the regions on the PC score scatterplot (Figure 4B). NC 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 ∑PCBs and dieldrin, which is not surprising because dieldrin made up a large percentage of the difference in ∑POP contributions seen between NC and W FL. In fact, W FL had higher average contributions of all pesticides (mirex, dieldrin, ∑chlordanes, ∑DDTs, and ∑toxaphenes) 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 Mississippi River watershed draining into the Gulf of Mexico, resulting in a higher proportion of pesticide exposure relative to more industrial PCB compounds.

A more detailed look at the PCB congener patterns revealed slight regional differences (Figure 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 contributions of PCB 118 and 153 were on average higher in W FL than the other two regions, whereas the opposite was true for PCB 138+163. The reason for these pattern differences is unknown but could be due to different PCB technical mixtures contaminating the foraging habitats of these females.

The PBDE pattern differences might be more interesting than those of the PCBs (Figure 5B). 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 had similar contributions of PBDEs 47, 100 and 154 on average. The E FL samples showed an intermediate pattern. Hites (2004) reviewed the literature of PBDE concentrations in a wide variety of biological samples mostly showing the typical pattern that was seen in W FL. Atypical patterns similar to the one in the NC samples has been noted recently for loggerhead plasma samples for NC (Carlson, 2006) and other reptile species, including freshwater turtles (*Sternotherus odoratus* and *Trachemys scripta troosti*) from Tennessee (Moss et al., 2009) and diamondback terrapins (*Malaclemys terrapin*) from New Jersey (Basile et al., 2011). This odd pattern is not species-specific, since loggerhead eggs from W FL have the typical pattern, but instead it seems to be geographically-specific to latitudes of 34 ^{o}N and higher in North America. This spatial difference could be due to releases of different PBDE formulations or different metabolic breakdown or elimination of congeners in different climates. Future studies should investigate these two possibilities.

Comparison of POP concentrations to other studies and toxic effects

Only one previous study of loggerhead egg POP concentrations is available to compare to our lipid-normalized concentrations. The average ∑PCB concentrations in loggerhead eggs from South Carolina (1188 ng/g lipid) (Cobb and Wood, 1997) fell right in line along the spatial gradient we observed. Those concentrations were much higher than E FL and less than NC. Eggs from the leatherback sea turtle nesting in E FL were measured for POPs recently (Stewart et al., in review) and have lower average concentrations of certain POP classes compared to the E FL loggerheads (mean ± standard deviation in ng/g lipid): 171 ± 150 ∑PCBs, 1.69 ± 0.12

mirex, 46.0 ± 33.4 ∑chlordanes, 37.9 ± 20.3 ∑DDTs, but similar concentrations of dieldrin (10.8 ± 6.8) and ∑toxaphenes (1.49 ± 0.63) and higher concentrations of ∑PBDEs 17.1 ± 12.6. The first four POP classes follow an expected trend based on trophic differences, because the jellyfish consuming leatherback sea turtle feeds lower on the food chain than omnivorous loggerhead. However, the latter three comparisons are surprising and likely due to differing foraging locations with the leatherback being capable of inhabiting water much further north than the loggerhead (Plotkin, 2003).

Currently, toxic thresholds are unknown for sea turtles. However, a significant negative correlation was seen between green sea turtle egg POP concentrations and hatchling mass:length ratio (van de Merwe et al., 2010) at concentrations ranging from (10.1 to 18.0) ng/g lipid (converted using the average percent lipid reported). The ∑POPs concentration in the current study ranged from 9.28 ng/g lipid to 6910 ng/g lipid, much higher than those reported in the green turtles from Malaysia. If the correlation observed in these green turtles extrapolated to higher concentrations, then loggerhead hatchlings off of all US southeastern regions could be at risk for poor body condition. Additionally, NC turtle egg ∑PCB, 4,4'-DDE, ∑chlordane, mirex and dieldrin concentrations fall within the range of concentrations measured in snapping turtles from Areas of Concern in the Lake Erie (de Solla and Fernie, 2004) but are lower than snapping turtle eggs from three highly contaminated sites in Lake Ontario where developmental abnormalities have been documented (Bishop et al., 1998). Without knowing the sensitivity of developing loggerhead sea turtles specifically to these compounds, it is difficult to impossible to determine the risk of POPs to their survival. 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-hatch, and sex ratios of the resulting hatchlings from the same nests.

Disclaimer

Certain commercial equipment or instruments are identified in the paper to specify adequately the experimental procedures. Such identification does not imply recommendations or endorsement by the NIST nor does it imply that the equipment or instruments are the best available for the purpose.

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Table 1. Loggerhead sea turtle nest location and sample size information.

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

*Number of individual yolk samples averaged from Alava et al. (2006).

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

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

%>RL = percent of nests with concentrations above the reporting limit; *n* = number of nests analyzed individually; SD = one standard deviation; NA = not available

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

Figure Legends

Figure 1. Location of loggerhead sea turtle egg sampling in relation to the four identified nesting recovery units in the U.S. (NMFS and FWS, 2008).

Figure 2. Regional differences in persistent organic pollutant concentrations (ng/g lipid) in loggerhead sea turtle egg yolk samples from nests laid in three regions. Mean and standard error are shown. W FL = western FL; E FL = eastern FL; NC = North Carolina. Different letters indicate significant differences among regions ($p < 0.016$).

Figure 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 was compiled for the Northern Recovery Unit from Bald Head Island, NC, and Wassaw Island, GA, nesting beaches (Hawkes et al. 2007; Plotkin and Spotila 2000). Tag return data from Melbourne Beach, FL in eastern FL came from Meylan et al. (1983). Satellite tracking data from Sarasota County, FL in western FL came from Girard et al. (2009).

Figure 4. Persistent organic pollutant (POP) patterns in loggerhead sea turtle egg yolk samples from nests laid in three regions. W FL = western FL; E FL = eastern FL; NC = North Carolina. A) Summed contaminant classes as a total of all POPs, data are mean and one standard deviation. B) Scatterplot of the first two principle component (PC) scores.

Figure 5. PCB (A) and PBDE (B) patterns in loggerhead sea turtle egg yolk samples from nests laid in three regions. W FL = western FL; E FL = eastern FL; NC = North Carolina, data are mean and one standard deviation. Only congeners with >1% of total within any region are shown.

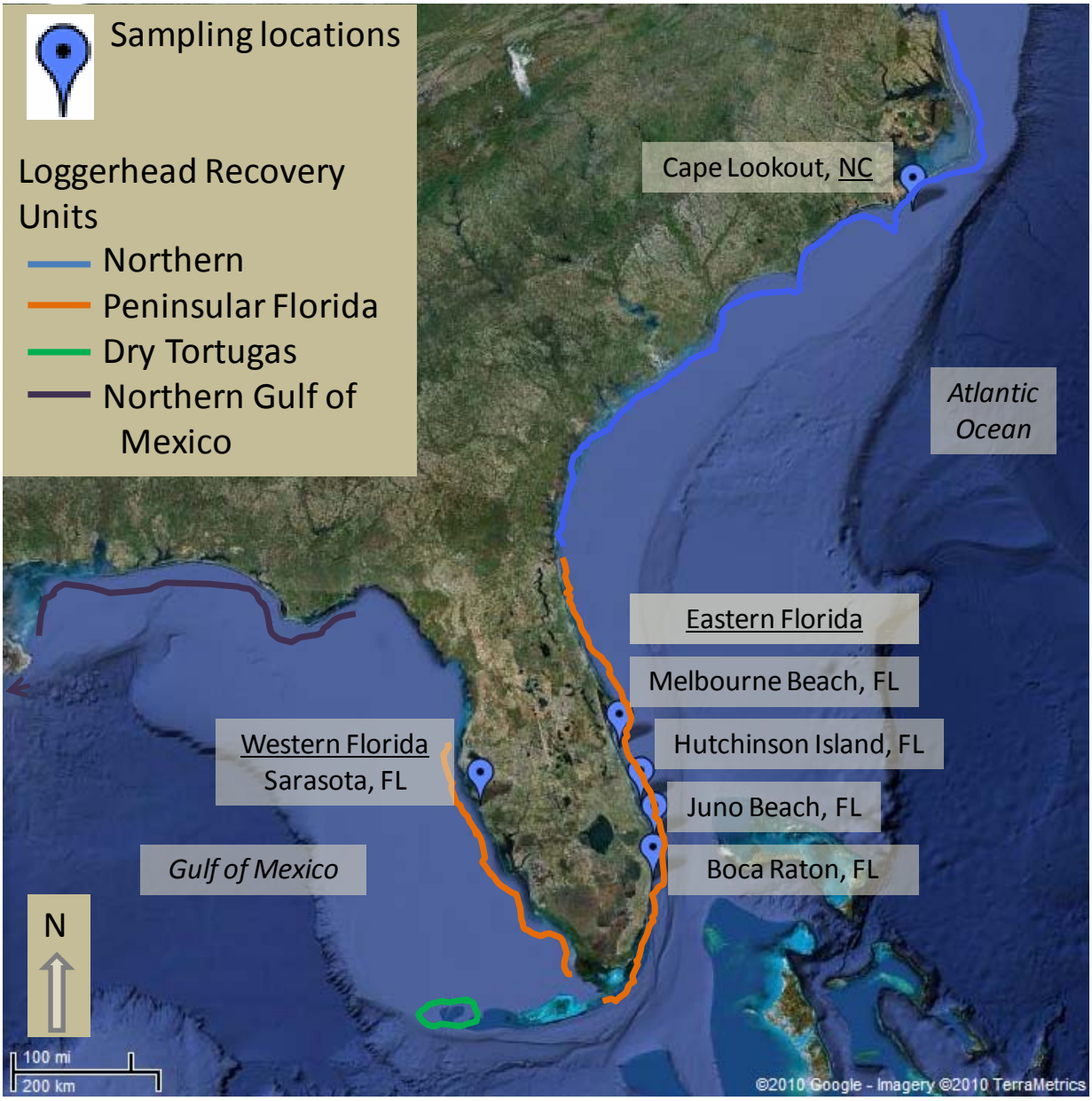
Sampling locations
Loggerhead Recovery Units
Northern
Peninsular Florida
Dry Tortugas
Northern Gulf of Mexico
Cape Lookout, NC
Atlantic Ocean
Eastern Florida
Melbourne Beach, FL
Hutchinson Island, FL
Juno Beach, FL
Boca Raton, FL
Western Florida
Sarasota, FL
Gulf of Mexico
N
100 mi
200 km
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Figure 1

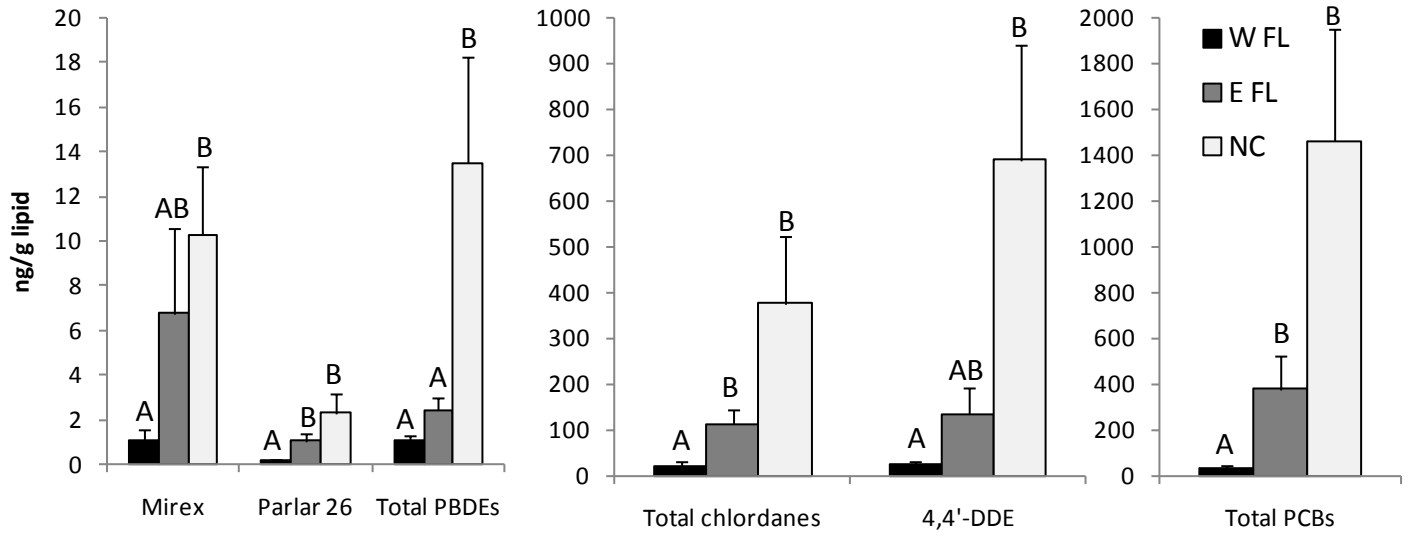

ng/g lipid
20
18
16
14
12
10
8
6
4
2
0
A
AB
B
A
B
B
A
A
B
Mirex
Parlar 26
Total PBDEs
1000
900
800
700
600
500
400
300
200
100
0
A
B
B
A
AB
B
Total chlordanes
4,4'-DDE
2000
1800
1600
1400
1200
1000
800
600
400
200
0
A
B
B
W FL
E FL
NC
Total PCBs

Figure 2

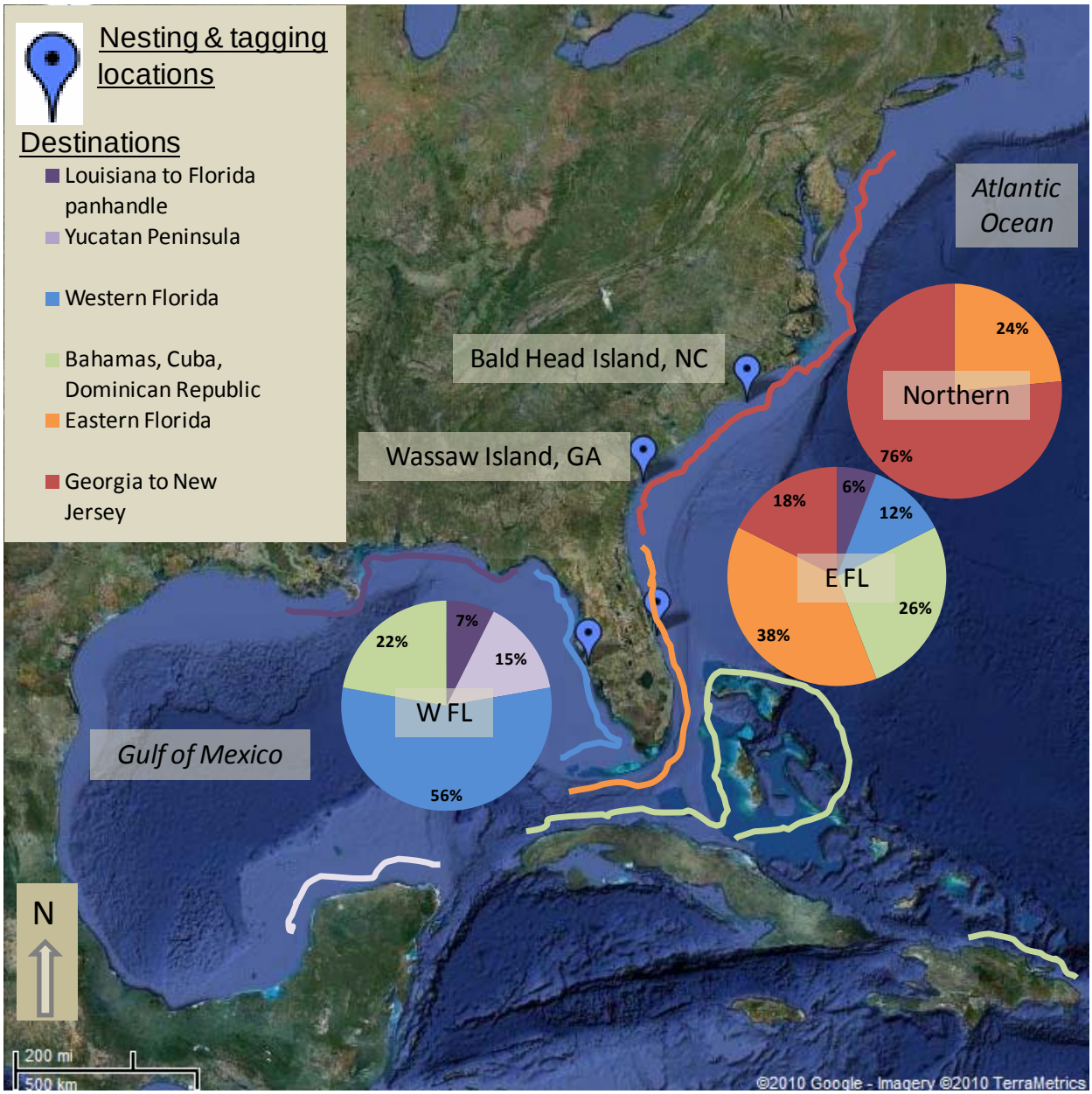

Nesting & tagging locations
Destinations
Louisiana to Florida panhandle
Yucatan Peninsula
Western Florida
Bahamas, Cuba, Dominican Republic
Eastern Florida
Georgia to New Jersey
Atlantic Ocean
Bald Head Island, NC
Wassaw Island, GA
Gulf of Mexico
Northern
24%
76%
E FL
6%
12%
26%
38%
18%
W FL
7%
15%
56%
22%
N
200 mi
500 km
©2010 Google - Imagery ©2010 TerraMetrics

Figure 3

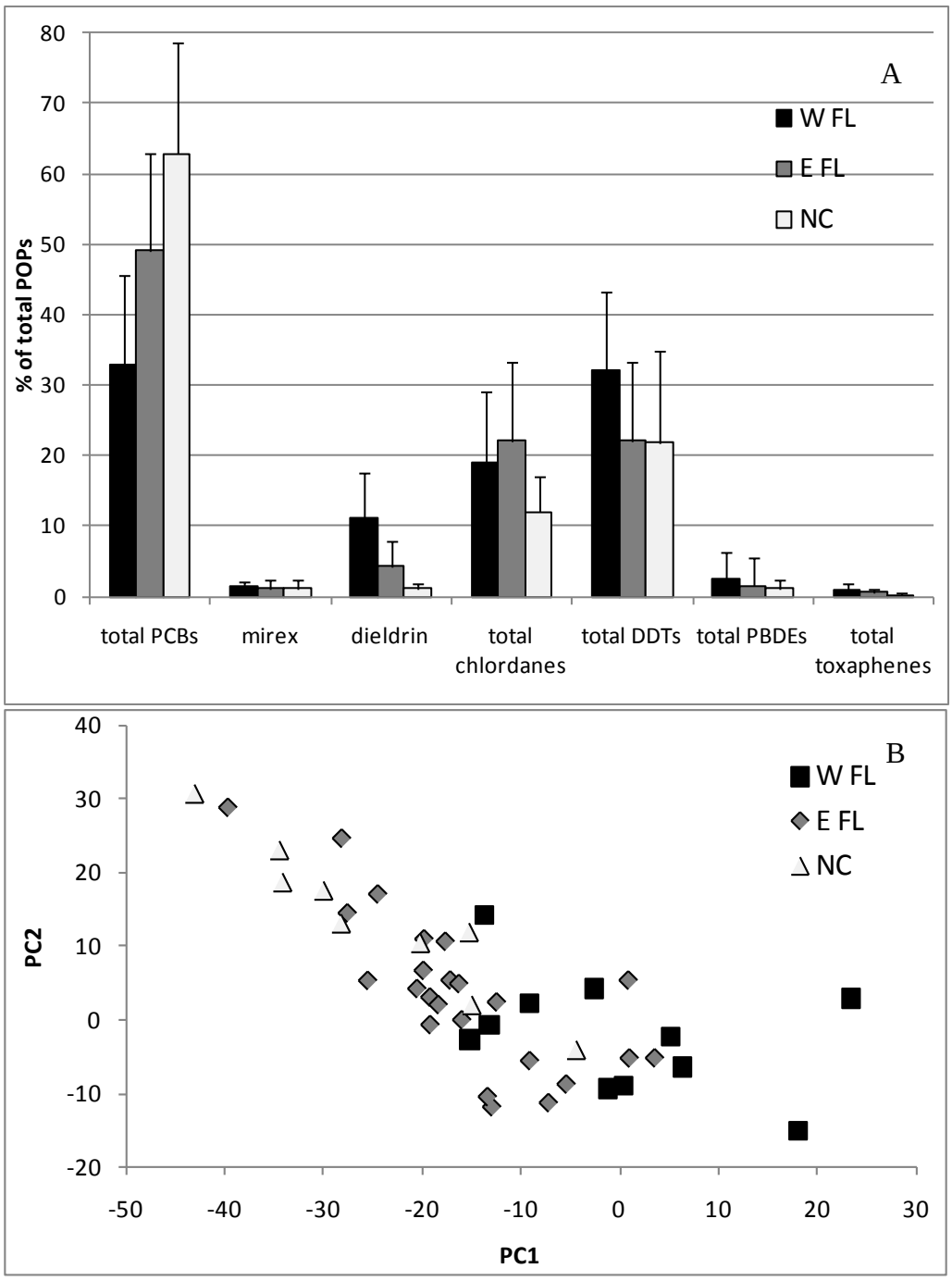
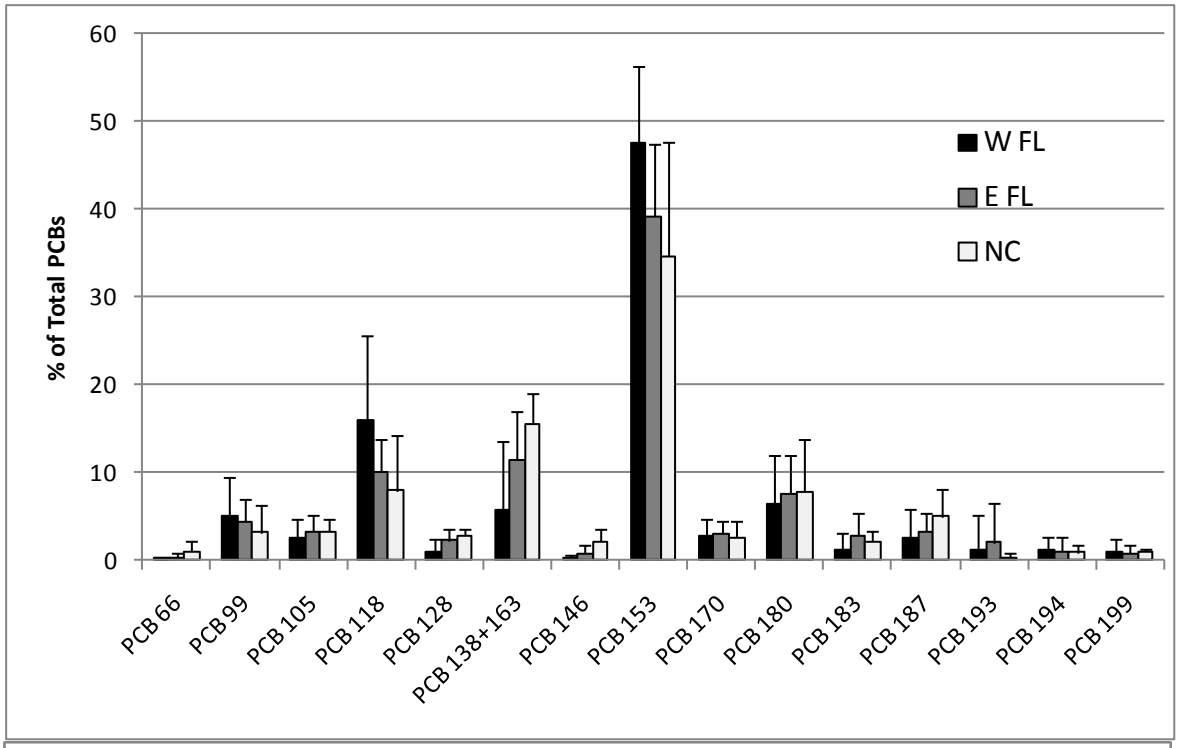

A
W FL
E FL
NC
% of total POPs
80
70
60
50
40
30
20
10
0
total PCBs
mirex
dieldrin
total chlordanes
total DDTs
total PBDEs
total toxaphenes
B
W FL
E FL
NC
PC2
PC1
40
30
20
10
0
-10
-20
-50
-40
-30
-20
-10
0
10
20
30

Figure 4


% of Total PCBs
60
50
40
30
20
10
0
W FL
E FL
NC
PCB 66
PCB 99
PCB 105
PCB 118
PCB 128
PCB 138+163
PCB 146
PCB 153
PCB 170
PCB 180
PCB 183
PCB 187
PCB 193
PCB 194
PCB 199

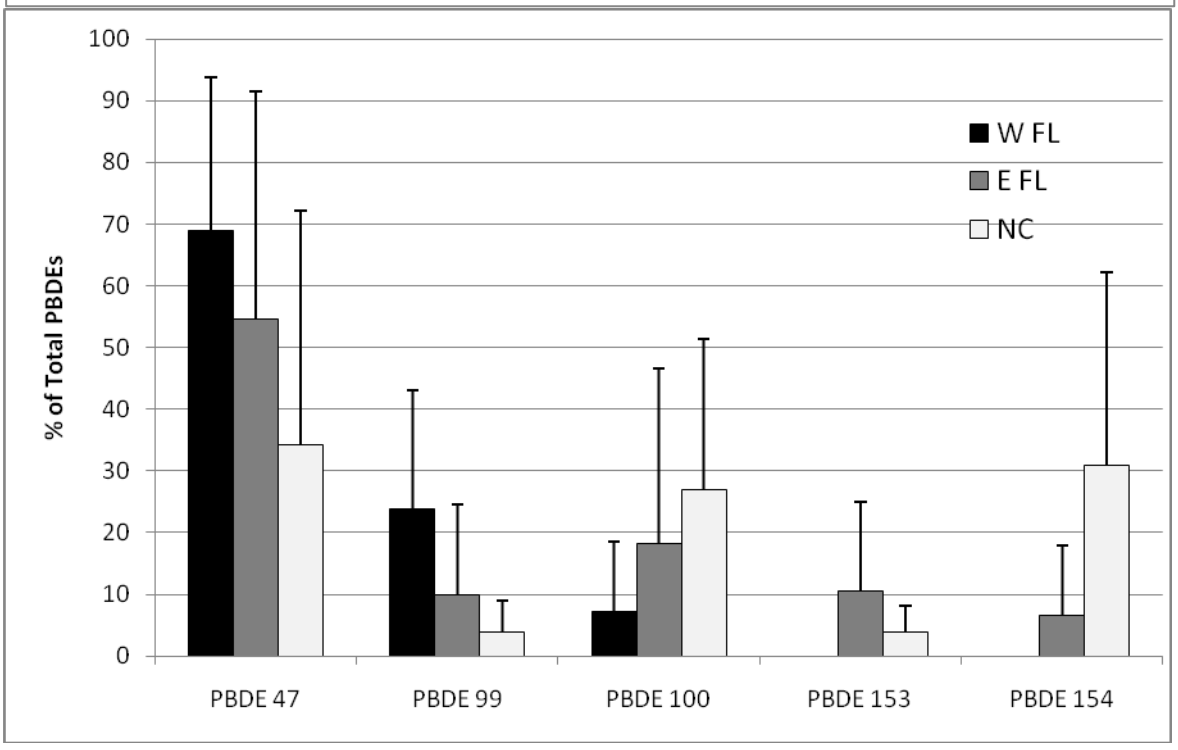

% of Total PBDEs
100
90
80
70
60
50
40
30
20
10
0
W FL
E FL
NC
PBDE 47
PBDE 99
PBDE 100
PBDE 153
PBDE 154

Figure 5