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# Perfluorinated Compounds in the Plasma of Loggerhead and Kemp's Ridley Sea Turtles from the Southeastern Coast of the United States

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Perfluorinated compounds (PFCs) have been measured in blood of humans and wildlife and are considered globally distributed contaminants. We examined 12 PFCs in the plasma of 73 loggerhead sea turtles (*Caretta caretta*) and 6 Kemp's ridley sea turtles (*Lepidochelys kempii*) captured from inshore waters of Core Sound, North Carolina (NC), and offshore waters of South Carolina, Georgia, and Florida (SC−FL). Perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) were the dominant compounds, with respective mean concentrations of 11.0 ng/mL and 3.20 ng/mL for loggerhead turtles and 39.4 ng/mL and 3.57 ng/mL for Kemp's ridley turtles. Mean PFOS concentrations were 2- to 12-fold higher than typical mean ΣPCB concentrations (∼5 ng/g wet mass) measured previously in sea turtle blood. More than 79% of the samples had detectable levels of perfluorocarboxylates (PFCAs) with 8−12 carbons, whereas only 17% or less of samples had detectable levels of PFCAs with 6 or 7 carbons. No samples had detectable levels of PFCAs with 4 or 5 carbons. In loggerhead turtles, ΣPFC concentrations were not influenced by sex ($p > 0.05$), but were higher in turtles captured from inshore waters of NC than in turtles from offshore waters of SC−FL ($p = 0.009$). A backward stepwise multiple regression model showed that ΣPFC concentrations were (1) significantly higher in Kemp's ridley turtles than loggerhead turtles ($p < 0.0001$), (2) higher in larger turtles ($p = 0.018$; carapace length used as a proxy for age), and (3) higher in turtles captured toward the north ($p = 0.006$). These findings suggest that bioaccumulation of PFCs in sea turtles is influenced by species, age, and habitat.

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## Introduction

Perfluorinated compounds (PFCs) are a broad class of chemicals that have a variety of applications, such as polymerization aids, stain repellents on carpets, textiles, leather, and paper products, and surfactants in fire-fighting foams, cosmetics, electronics, plastics, and medical devices (reviewed by *1*, *2*). Perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the most commonly measured PFCs in environmental samples. While both compounds have direct uses, they are also considered final degradation products of other PFC precursors (*3*, reviewed by *2*). PFCs persist in the environment because of the strength of the carbon−fluorine bond, and they have been measured in wildlife and human tissues around the globe (*4*, *5*). PFOS was measured up to 59.5 μg/g in mink liver (*6*), and its concentrations increased over the past two decades in ringed seal liver samples from Greenland (*7*), bird eggs from the Baltic Sea (*8*), and lake trout from Lake Ontario (*9*).

Although studies have examined PFC levels in several wildlife species, including marine animals such as marine mammals and sea birds (*4*, *10*−*12*), no study reports the occurrence of PFCs in sea turtles. Sea turtles face many human impacts, and all species of sea turtles found in U.S. coastal waters are protected by the U.S. Endangered Species Act. The loggerhead sea turtle (*Caretta caretta*) is designated as a threatened species, and, while some populations are recovering, the northern nesting subpopulation (nesting from northeastern FL to NC) may be declining (*13*). The Kemp's ridley sea turtle (*Lepidochelys kempii*) is the most endangered of all sea turtle species. Monitoring the concentrations of PFCs in sea turtle tissues is important for many reasons. PFC exposure in laboratory-tested animals produces toxic effects, including peroxisome proliferation, tumor production, liver damage, and alterations in cholesterol levels, steroid levels, mitochondrial bioenergetics, and lipid metabolism (reviewed by *14*). Because other persistent contaminants, such as organochlorine compounds and mercury, correlate with indicators of health in sea turtles (*15*−*17*), PFCs may also be a concern for sea turtle health.

The unique life history traits of sea turtles provide the ability to answer interesting questions about bioaccumulation of marine contaminants. As juveniles and again as adults, sea turtles migrate great distances. For example, hatchling loggerhead sea turtles leaving Florida beaches cross the Atlantic Ocean feeding omnivorously in the pelagic zone for approximately 7−10 years before returning to U.S. coastal areas to feed on benthic invertebrates during a second, more neritic (near-shore habitat with water depth < 200 m) juvenile stage (*18*). Sea turtles in the pelagic juvenile stage may indicate contamination of an entire ocean basin because they feed in areas distant from continental influences. However, juvenile loggerhead turtles in the second, more neritic juvenile stage show site fidelity in their summer foraging areas (*19*), and therefore they may reflect more local, coastal contamination. Sea turtles are also long-lived species; for example, loggerhead turtles reach maturity at 20 years of age or older (*20*). This long lifespan allows for long-term bioaccumulation of contaminants. While providing the first baseline PFC concentrations in sea turtles, the current study also explores the influence of life history traits on PFC concentrations, including a species comparison, gender differences, bioac-

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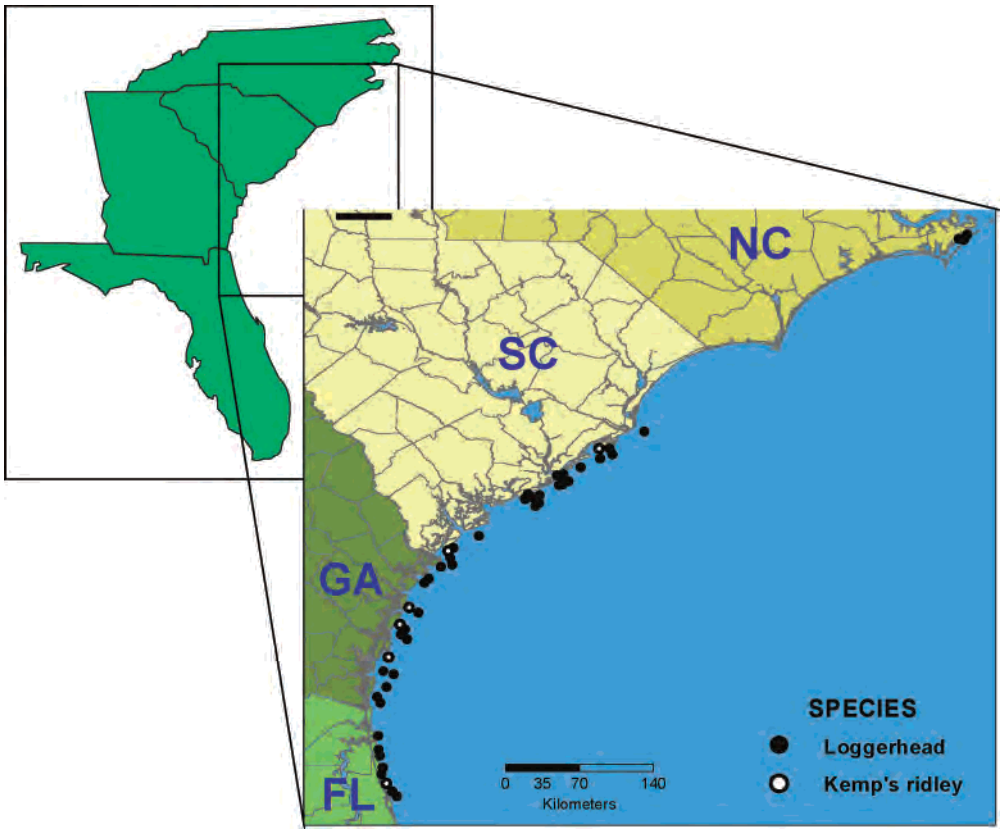


**FIGURE 1. Capture locations of loggerhead and Kemp's ridley sea turtles. Six loggerhead turtles were captured from Core Sound, NC, and 67 loggerheads and 6 Kemp's ridley turtles were captured in offshore waters of SC, GA, and northeastern FL.**

cumulation with carapace length (used as a proxy for age), and a geographical comparison.

## Materials and Methods

**Sampling.** Sixty-seven free-ranging loggerhead sea turtles and six Kemp's ridley sea turtles were captured in offshore waters of SC, GA, and northeastern FL during June and July 2003 (29.9° N to 34.8° N). These turtles were captured in trawl nets deployed without turtle excluder devices for 30 min at randomly selected stations within 8 miles of the coast. An additional 6 loggerhead turtles were captured as by-catch from a pound net fishery located in Core Sound, NC, in June 2003. Capture locations are shown in Figure 1. All turtles were tagged, measured, and released near their capture location. Straight carapace length (SCL), measured from the nuchal notch to the most posterior marginal notch, ranged from 53.9 to 92.7 cm (mean 66.0 cm) for loggerhead turtles and from 37.5 to 54.1 cm (mean 46.9 cm) for Kemp's ridley turtles. On the basis of the SCLs, all turtles were sexually immature and the loggerhead turtles were categorized in the second, more neritic juvenile stage with the exception of one adult male loggerhead.

Blood was collected as described in Keller et al. (*16*) within 15 min of handling the turtles and was stored at 4 °C until processing. Plasma from one tube of blood was removed within 6 h and stored at −80 °C until analyzed for testosterone concentrations using a radioimmunoassay, which was previously validated (with concurrent laparoscopic examination of gonads) as a reliable method to determine the sex of sea turtles (*21*). The sex ratios of females/males/unknowns were 43:17:13 for loggerhead turtles and 4:1:1 for Kemp's ridley turtles. Plasma from another blood tube was transferred within 36 h of blood collection in a sterile tissue culture hood using sterile polyethylene pipets into polypropylene vials and stored at −80 °C for PFC measurements.

**TABLE 1. Perfluorinated Compounds Measured in Sea Turtle Plasma Samples**

| compound | abbreviation | MDL[a] (pg/mL) | MRM[b] transitions (*m*/*z*) |
|---|---|---|---|
| **perfluorocarboxylates (PFCAs)** | | | |
| perfluorobutanoic acid | PFBuA | 50 | 212.8 → 168.8 |
| perfluoropentanoic acid | PFPeA | 50 | 262.8 → 218.7 |
| perfluorohexanoic acid | PFHxA | 100 | 312.8 → 268.8 |
| perfluoroheptanoic acid | PFHpA | 100 | 362.8 → 318.8 |
| perfluorooctanoic acid | PFOA | 100 | 413.0 → 368.7 |
| perfluorononanoic acid | PFNA | 100 | 462.7 → 418.8 |
| perfluorodecanoic acid | PFDA | 100 | 512.8 → 468.8 |
| perfluoroundecanoic acid | PFUnDA | 50 | 563.0 → 519.0 |
| perfluorododecanoic acid | PFDoDA | 50 | 612.7 → 568.8 |
| **perfluoroalkanesulfonates/amides (PFSs)** | | | |
| perfluorohexanesulfonate | PFHxS | 50 | 398.7 → 79.7 |
| perfluorooctanesulfonate | PFOS | 50 | 498.6 → 79.7 |
| perfluorooctanesulfonamide | PFOSA | 50 | 497.7 → 77.7 |
| perfluorobutanesulfonate[c] | PFBS | | 298.7 → 79.7 |

[a] MDL = method detection limit. [b] MRM = multiple reaction monitoring. [c] Surrogate standard.

**PFC Analysis.** Names and abbreviations of the 12 PFCs measured are listed in Table 1. PFOSA and potassium salts of PFHxS and PFBS were donated by the 3M Company (St. Paul, MN). Potassium salt of PFOS was from Tokyo Chemical Industries (Portland, OR). PFBuA and PFNA were from Avocado Research Chemicals, Ltd (Lancashire, UK). PFPeA, PFHpA, PFDA, PFUnDA, and PFDoDA were from Fluorochem Ltd (Derbyshire, UK). PFHxA was from Wako Pure Chemical Industries (Tokyo, Japan). PFOA was from Strem Chemicals, Inc (Newburyport, MA). The stated purity of all compounds was ≥95%.

Sample extraction and PFC measurements were performed at the Wadsworth Center (Albany, NY) and the

National Institute of Advanced Industrial Science and Technology (Ibaraki, Japan) using slight modifications of an ion-pairing method (*22*). Briefly, plasma samples were spiked with 15 ng of PFBS, chosen as a surrogate standard for the following several reasons: (1) no $^{13}C$-labeled standards were available at this time, (2) PFBS was not detected in seawater or a variety of species collected in 2002 (*23*), (3) accumulation of PFBS from water could not be detected in fish during an exposure study (*24*), and (4) 3M announced that it was replacing PFOS in Scotchguard with PFBS near the same time as this sampling effort. For these reasons, PFBS was unlikely to be found in the sea turtle samples. Approximately 0.5−1.0 mL of plasma, 1 mL of 0.5 mol/L tetrabutylammonium hydrogen sulfate (pH 10), and 2 mL of 0.25 mol/L sodium carbonate were thoroughly mixed in a polypropylene tube. The mixture was shaken for 20 min with 5 mL of methyl-*tert*-butyl ether (MTBE). After centrifugation, exactly 4 mL of the MTBE extract was transferred to a second polypropylene tube. The MTBE extraction was repeated once more. The extract was evaporated under nitrogen, reconstituted in methanol, vortexed for 30 s, passed through a 0.2-$\mu$m nylon mesh filter, and brought to 1 mL volume in an autosampler vial.

Analyte separation was performed using an Agilent 1100 HPLC (Palo Alto, CA). A 10-$\mu$L aliquot of each extract and calibration standard was injected onto a 50 × 2.1 mm (5 $\mu$m) Keystone Betasil $C_{18}$ column with a 2 mmol/L ammonium acetate/methanol mobile phase starting at a volume fraction of 10% methanol. At a flow rate of 300 $\mu$L/min, the gradient was increased to 100% methanol at 10 min before reverting to original conditions at 12 min. Column temperature was maintained at 20 °C. For quantitative determination, the HPLC system was interfaced to a Micromass (Beverly, MA) Quattro II atmospheric pressure ionization tandem mass spectrometer operated in the negative electrospray ionization mode. Instrumental parameters were optimized to transmit the $[M-K]^-$ ion before fragmentation to one or more product ions. Cone voltage and collision energies were optimized for each analyte, and ranged from 35 to 90 V and 7 eV to 35 eV, respectively. Data were acquired by tandem mass spectrometry using multiple reaction monitoring (MRM). When possible, multiple daughter ions were monitored for confirmation, but quantification was based on a single product ion (Table 1). In all cases, the capillary was held at 1 kV. Desolvation temperature and gas flow were kept at 400 °C and 685 L/h, respectively.

**Quality Assurance.** External standards used for the calibration curve included 0.1, 0.5, 1, 10, and 20 ng/mL. Concentrations as low as 0.010 ng/mL were also injected and produced signal-to-noise ratios of ∼10 for PFOS and PFOA. Samples were diluted or concentrated to a known volume and re-injected, when necessary, to obtain areas that fell within the calibration range. Quantification was not based on relative response factors with the PFBS standard. Minimum detection limits (MDLs shown in Table 1) were calculated based on the procedural blanks, run with every 20 samples, and represented at least 3× the concentration calculated in the blanks with corrections for sample volume and dilution factors. A signal-to-noise ratio of three was also required to be above the MDL. Due to improvements made to eliminate procedural and instrumental sources of contamination (*25*), typical blanks contained only trace levels of PFOS and PFOA (<10 pg/mL and <50 pg/mL, respectively). Methanol was injected between samples to avoid potential injection-port carryover. Recoveries of PFBS ranged from 72% to 139%. Subsamples of loggerhead plasma ($n = 5$ run in duplicate) were spiked with 10 ng of each target compound. Recoveries ranged from 70% to 120% in these matrix spikes and matrix spike duplicates. The reported concentrations were not corrected for recovery, blank contamination, or stated purity of standards.

**Statistics.** Each statistical test was performed for PFOA, PFNA, PFDA, PFUnDA, PFDoDA, ΣPFCA, PFHxS, PFOS, and ΣPFC concentrations, which are the compounds that were detectable in most samples. When concentrations were not normally or log-normally distributed, nonparametric tests were used. When possible, parametric tests were performed using log-transformed concentrations, and equality of variances was confirmed using Bartlett's homogeneity of variance tests. Compounds <MDL were set equal to half the MDL prior to running statistical tests. When summing groups of compounds, values that were <MDL were set equal to zero. To compare among female, male, and unknown-sex loggerhead turtles, the Kruskal−Wallis test or analysis of variance was used. To compare loggerhead turtles captured from the two sampling locations (inshore NC versus offshore SC−FL), Wilcoxon or *t*-tests were used. Backward stepwise multiple regressions were performed on log-transformed concentrations in turtles sampled in the offshore project with species, SCL, and latitude of capture location as independent variables. The residuals of each model were normally distributed. NC samples were excluded from the multiple regression analyses to avoid confounding the latitudinal assessment by differences between inshore (NC) and offshore (SC−FL) PFC concentrations. All statistical analyses were performed using JMP 4.0.2 (SAS Institute, Cary, NC).

## Results and Discussion

A representative chromatogram of a loggerhead turtle plasma sample containing average PFC concentrations is shown in the Supporting Information. The early shoulder on the PFOS peak is likely branched isomers of PFOS (*22*). PFOA and PFOS were found at the highest concentrations and were detected in all samples (Table 2). PFNA, PFDA, PFUnDA, PFDoDA, and PFHxS were detected in more than 76% of the loggerhead samples and in all Kemp's ridley samples. PFHpA was detected in 12% and 17% of the loggerhead and Kemp's ridley samples, respectively. PFHxA was detected in 8% of the loggerhead samples but not in Kemp's ridley samples. PFOSA was detected in only one loggerhead sample and none of the Kemp's ridley samples. PFBuA and PFPeA were not detected in any samples.

The ΣPFC concentrations were compared to the total polychlorinated biphenyl (ΣPCB) concentrations previously measured in the blood of these two species (*26*) after converting the ΣPCB concentrations to a wet-mass basis. The mean ΣPFC concentration was approximately 3× higher than ΣPCB concentrations (5.56 ng/g) in loggerhead turtles and 15× higher than ΣPCB concentrations (3.33 ng/g) in Kemp's ridley turtles. PFOS concentrations alone were 2- to 12-fold higher than mean ΣPCB blood concentrations. A previous study found that PFOS concentrations were higher than any one individual PCB congener in polar bear liver samples (*27*), but to our knowledge the current study is the first to report that PFOS concentrations exceed total PCB concentrations.

Sea turtle PFOS concentrations (geometric mean: 6.39 ng/mL for loggerhead turtles and 35.5 ng/mL for Kemp's ridley turtles) are comparable with human plasma levels. For example, the geometric mean concentration of PFOS in Americans was 34.9 ng/mL (*14*). PFOS concentrations in sea turtle plasma are also similar to Laysan and black-footed albatrosses from the Midway Atoll (18 ng/mL) and are on the same order of magnitude as snapping turtles from Lake St. Clair, Michigan (72 ng/mL), but are lower than yellow-blotched map turtles from Mississippi (190 ng/mL) and bald eagles from the U.S. Midwestern states (360 ng/mL) (*4*). PFOS concentrations were 2 orders of magnitude lower in sea turtle plasma compared to the geometric mean in plasma of bottlenose dolphins captured from Charleston, SC (1171 ng/

**TABLE 2. Perfluorinated Compound Concentrations (pg/mL) in Loggerhead and Kemp's Ridley Sea Turtle Plasma**

| | loggerhead sea turtles | | | | Kemp's ridley sea turtles | | | |
|---|---|---|---|---|---|---|---|---|
| compound | mean (SD)[a] pg/mL | median (pg/mL) | range (pg/mL) | $n >$ MDL[b] | mean (SD)[a] pg/mL | median (pg/mL) | range (pg/mL) | $n >$ MDL[b] |
| PFBuA | <50 | <50 | | 0 | <50 | <50 | | 0 |
| PFPeA | <50 | <50 | | 0 | <50 | <50 | | 0 |
| PFHxA | 9.27 (31.4) | <100 | <100 to 124 | 6 | <100 | <100 | | 0 |
| PFHpA | 21.1 (64.1) | <100 | <100 to 358 | 9 | 16.9 (41.3) | <100 | <100 to 101 | 1 |
| PFOA | 3200 (1490) | 2950 | 493 to 8140 | 73 | 3570 (554) | 3460 | 2770 to 4250 | 6 |
| PFNA | 298 (588) | 172 | <100 to 4420 | 58 | 1654 (2400) | 710 | 258 to 6500 | 6 |
| PFDA | 515 (858) | 247 | <100 to 6350 | 67 | 2370 (1250) | 2390 | 664 to 4300 | 6 |
| PFUnDA | 355 (515) | 200 | <50 to 2950 | 66 | 1420 (789) | 1420 | 333 to 2670 | 6 |
| PFDoDA | 181 (151) | 153 | <50 to 827 | 69 | 462 (150) | 544 | 241 to 586 | 6 |
| ΣPFCAs | 4580 (2910) | 3940 | 561 to 21600 | 73 | 9490 (3000) | 9070 | 5740 to 13700 | 6 |
| PFHxS | 155 (201) | 99.0 | <50 to 1080 | 56 | 253 (89.5) | 252 | 119 to 382 | 6 |
| PFOS | 11000 (17200) | 5450 | 1400 to 96800 | 73 | 39400 (17100) | 41900 | 13800 to 60200 | 6 |
| PFOSA | 0.837 (7.15) | <50 | <50 to 61.1 | 1 | <50 | <50 | | 0 |
| ΣPFSs | 11200 (17300) | 5620 | 1400 to 97000 | 73 | 39700 (17100) | 42200 | 13900 to 60600 | 6 |
| ΣPFCs | 15800 (19100) | 9720 | 3430 to 106000 | 73 | 49200 (17700) | 51200 | 19600 to 67900 | 6 |

[a] SD = standard deviation. [b] Number of samples that had concentrations greater than the minimum detection limit (MDL) out of 73 loggerhead and 6 Kemp's ridley turtles.

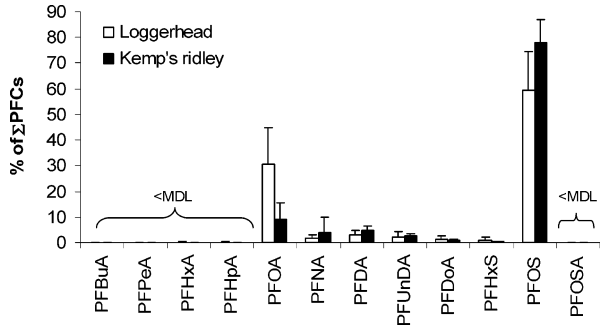


**FIGURE 2. Patterns of individual PFCs as a percent of ΣPFCs in the plasma of loggerhead and Kemp's ridley sea turtles. Compounds are indicated that were found <MDL in the majority of the samples. Error bars represent one standard deviation.**

g) (*12*), even though both species were inhabiting the same general area. The most likely reasons for this large difference are that sea turtles feed on a lower trophic level and probably consume less food on a body mass basis than homeothermic dolphins, and the turtles were captured farther offshore than the dolphins.

The patterns of individual PFCs as a percent of ΣPFCs are shown in Figure 2. PFOA and PFOS were the dominant compounds in both species. In the loggerhead turtles, PFOS and PFOA, on average, represented 60% and 31% of the ΣPFC concentration, respectively. PFOS represented a higher percentage (78%) and PFOA represented a lower percentage (9%) in the Kemp's ridley turtles compared to loggerhead turtles.

The patterns observed in sea turtles share some similarities and dissimilarities to patterns previously noted in other species from other locations. Similarly, most studies show that PFOS concentrations are generally higher than individual perfluorocarboxylic acids (PFCAs) or ΣPFCAs (*6*, *9*, *12*, *14*). Few to no turtles had detectable levels of PFCAs with 7 or fewer perfluoroalkyl carbons (Table 2), which is supported by an exposure study showing greater bioconcentration of longer chain PFCAs and no accumulation of those with less than 7 carbons (*24*). Martin et al. (*9*) demonstrated that species using different foraging tactics in Lake Ontario exhibited different PFCA patterns. PFCA concentrations generally decreased with increasing perfluoroalkyl carbon chain lengths in the benthic-feeding amphipod (*Diporeia hoyi*) and sculpin (*Cottus cognatus*), whereas the primarily planktonivorous species, such as a crustacean (*Mysis relicta*) and alewife (*Alosa pseudoharengus*), showed little to no relationship between PFCA concentration and chain length. The primarily benthic-feeding loggerhead and Kemp's ridley turtles exhibited a decreasing trend in PFCA concentration with increasing chain length, similar to the benthic-feeding species in Lake Ontario.

In several species of Arctic fish, birds, and mammals, PFOA generally contributes a minor percentage to the ΣPFCA concentration, and PFCAs containing an odd number of perfluoroalkyl carbons are found at higher concentrations than the corresponding shorter, even number PFCA (*27*). In contrast, PFOA contributed a large component of the ΣPFCAs in sea turtles (41% of ΣPFCAs in Kemp's ridley turtles and 75% in loggerhead turtles) and the opposite odd/even pattern was observed in the turtles. PFOA concentrations were significantly higher than PFNA ($p < 0.0001$), as was PFDA compared to PFUnDA ($p < 0.0001$) (Table 2). Global transport processes may alter the pattern of PFCAs observed in the Arctic compared to more temperate regions. Ellis et al. (*3*) suggest that the primary route of PFCA contamination in the Arctic is through atmospheric transport of more volatile fluorotelomer alcohols (FTOHs) that are degraded in the atmosphere to PFCAs. Since 8:2 FTOH degrades into PFOA and PFNA in equal amounts, the authors speculate that the higher concentrations of PFNA, the odd chain-length compound, in Arctic mammals results from its greater bioaccumulation potential compared to PFOA. The processes of PFCA contamination in temperate, coastal regions of the United States are undoubtedly more complex because of closer proximity to manufacturing and use and from greater influence of less volatile PFCs transported by water. Therefore, it is not surprising that the sea turtles have a different pattern than Arctic animals. More studies are needed to address bioaccumulation, biotransformation, physical/chemical degradation, and global transport pathways of PFCs.

The influences of species, sex, age, and geographical location on PFC concentrations were examined using various statistical tests, and the sample choice for each test and *p*-values are summarized in Table 3. Species differences were observed (Figure 3). Kemp's ridley turtles had significantly higher concentrations of PFNA, PFDA, PFUnDA, PFDoDA, ΣPFCAs, PFHxS, PFOS, and ΣPFCs than loggerhead turtles. However, PFOA concentrations were not different between species. A species difference has been noted previously for ΣPCBs in the adipose tissue of these two species (*26*) and is likely due to either Kemp's ridley turtles feeding at a slightly

**TABLE 3. Summary of Statistical Tests and Findings Examining the Influence of Life History Traits on Perfluorinated Compound Concentrations in Loggerhead and Kemp's Ridley Sea Turtle Plasma[a]**

| | Data Selection for Statistical Test | | | *p*-Value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| trait | species | project location | statistical test | PFOA | PFNA | PFDA | PFUnDa | PFDoDA | ΣPFCAs | PFHxS | PFOS | ΣPFCs |
| sex | loggerhead only | both included | Kruskal–Wallis | 0.0678[b] | 0.2306 | 0.1007 | 0.0509 | 0.9530 | 0.0895 | 0.2153 | 0.1998 | 0.1241 |
| project location | loggerhead only | compare NC to SC–FL | Wilcoxon | 0.6677[c] | 0.1023 | **0.0148**[c] | **0.0001**[c] | **0.0198** | 0.0859 | 0.0821 | **0.0078** | **0.0093** |
| species | both included in model | SC–FL only | multiple regression | >0.1000[d] | **<0.0001** | **<0.0001** | **<0.0001** | **<0.0001** | **0.0002** | **0.0009** | **<0.0001** | **<0.0001** |
| turtle length | both included in model | SC–FL only | multiple regression | >0.1000 | **0.0147** | **0.0220** | >0.1000 | >0.1000 | >0.1000 | **0.0164** | **0.0109** | **0.0177** |
| capture latitude | both included in model | SC–FL only | multiple regression | >0.1000 | **0.0341** | **0.0002** | **0.0144** | >0.1000 | 0.0885 | >0.1000 | **0.0041** | **0.0064** |
| | | | ANOVA for entire multiple regression model | 0.5955 | **<0.0001** | **<0.0001** | **<0.0001** | **0.0003** | **0.0004** | **0.0041** | **<0.0001** | **<0.0001** |

[a] Bolded *p*-values were considered statistically significant ($p < 0.05$). [b] An analysis of variance was performed on log-transformed concentrations rather than a Kruskal–Wallis test. [c] A parametric *t*-test was performed on log-transformed concentrations rather than a Wilcoxon test. [d] Values shown as >0.1000 indicate that the backward stepwise multiple regression model eliminated that particular model effect.

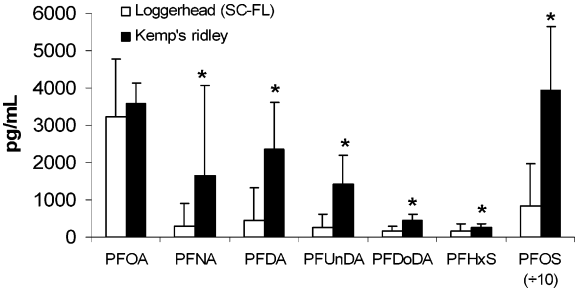


**FIGURE 3. Species comparison of loggerhead and Kemp's ridley sea turtle plasma PFC concentrations. Compounds that were detectable in most samples are shown. Error bars represent one standard deviation (SD). The mean and SD of PFOS concentrations were divided by 10 to match the PFCA scale. An asterisk indicates a significant difference between species ($p < 0.05$ for species as a model effect in backward stepwise multiple regressions).**

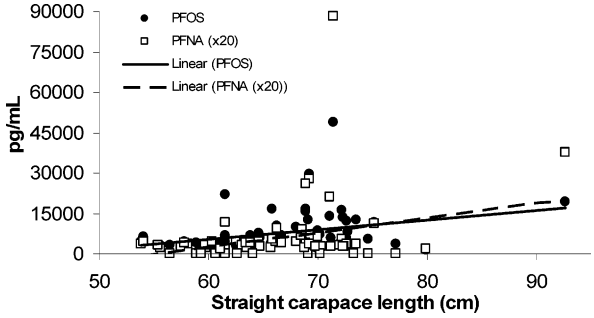


**FIGURE 4. Scatterplot of loggerhead sea turtle straight carapace length (used as a proxy for age) versus plasma PFOS and PFNA concentrations. Trendlines are drawn to demonstrate the positive relationships. Statistical significance was determined using straight carapace length as a model effect in a backward stepwise multiple regression ($p < 0.05$). The PFNA concentrations were multiplied by 20 to match the PFOS scale.**

higher trophic level than loggerhead turtles or inhabiting near-shore waters for most of their juvenile phase. Future studies should compare PFC concentrations along with stable isotopes in turtle species found on very different trophic levels to examine biomagnification. For example, the omnivorous species chosen for this study could be compared to the herbivorous green sea turtle (*Chelonia mydas*).

No significant sex differences were observed among male, female, and unknown-sex loggerhead turtles for any compound measured (Table 3). Sex differences were not expected because these turtles were juveniles, so the females had no opportunity to transfer compounds to eggs. Maternal transfer of PFCs should be investigated in future sea turtle studies, because studies have shown evidence of placental transfer in humans (*28*) and oviparous transfer in birds (*4*, *29*) and in fish (*6*). A recent study measuring PFOS in snapping turtle plasma also provides evidence of reproductive transfer in a reptile, as adult males had 22× higher concentrations than adult females (*6*). Strong evidence for oviparous transfer was recently demonstrated in a dietary PFOS exposure experiment with bobwhite quail (*29*). Exposed females produced eggs with high levels of PFOS (mean = 62 μg/g), and the adult female serum and liver concentrations were 16–18 times lower than adult males exposed to the same dose.

Bioaccumulation of PFCs through age was investigated using multiple regression models using SCL as a proxy for age. Turtle length significantly influenced the concentrations of PFNA, PFDA, PFHxS, PFOS, and ΣPFCs, but not of PFOA, PFUnDA, PFDoDA, or ΣPFCAs (Table 3). Figure 4 demonstrates that larger, presumably older loggerhead turtles had higher concentrations of PFNA and PFOS. If the adult male (>90 cm SCL) is excluded from the statistical analyses, turtle length no longer significantly influenced PFC concentrations (except for PFOS, $p = 0.038$), suggesting that this turtle strongly influenced the models. Another outlying sample is a juvenile female (71.4-cm SCL) that contained the highest concentrations of certain PFCs in the SC–FL loggerhead samples (i.e., 48 802 pg/mL of PFOS, 4421 pg/mL of PFNA, 71 500 pg/mL of ΣPFCs). If this turtle is excluded from the multiple regression, all relationships of turtle length with PFC concentrations remained significant ($p \leq 0.025$). It is important to note the limitation of using length as a proxy for age. Currently, noninvasive methods are not available to determine the age of live sea turtles; therefore, turtle size is the only estimate of age. While growth rates can be plastic resulting in same age turtles of very different sizes (*30–31*), carapace length has been shown to be significantly related to age in known-age turtles and to age determined using skeletochronology (*20*, *30–31*). These studies suggest that turtle size can be used as an approximation for age. Future analyses should expand this sample set to include more adults to increase the range of ages represented.

PFOS in the current study correlated with turtle length, but PFOA did not. Studies examining bioaccumulation of

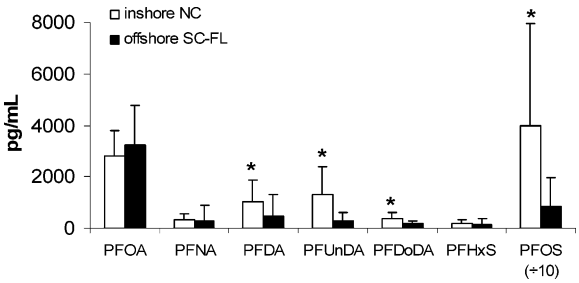


**FIGURE 5. Geographical comparison of plasma concentrations of PFCs between loggerhead turtles captured from inshore waters of NC and loggerhead turtles captured from offshore waters of SC to northeastern FL. Compounds that were detectable in most samples are shown. Error bars represent one standard deviation (SD). The mean and SD of PFOS concentrations were divided by 10 to match the PFCA scale. An asterisk indicates a significant difference between locations ($p < 0.05$ in Wilcoxon or *t*-tests).**

PFCs through age in other species report inconsistent results. Some studies have observed increasing trends of PFOS with age (*32*), while others have observed decreasing (*12*) or no significant trends (*5*, *11*, *14*, *23*, *28*). Fewer studies have addressed bioaccumulation of PFOA through age and have found either a decreasing trend (*12*) or no trend (*5*, *14*). Collectively, these results suggest that PFOS may accumulate with age in some species while PFOA does not appear to do so. Future studies with other species should investigate age-related bioaccumulation of the higher carbon chain PFCAs (i.e., PFNA and PFDA) that significantly related to length in the loggerhead turtles.

Capture location strongly influenced the concentrations of certain PFCs (Table 3). Loggerhead turtles captured in the inshore waters of NC had significantly higher concentrations of PFDA, PFUnDA, PFDoDA, PFOS, and ΣPFCs, but not of PFOA, PFNA, ΣPFCAs, or PFHxS compared to turtles captured in the offshore site of SC−FL (Figure 5). The geographical differences may be explained by NC turtles feeding in inshore waters closer to human activities versus the SC−FL turtles inhabiting offshore waters. However, strong positive relationships between capture latitude and PFNA, PFDA, PFUnDA, PFOS, and ΣPFC (not PFOA, PFDoDA, ΣPFCAs, or PFHxS) concentrations within the offshore SC−FL samples (Table 3) suggest that turtles captured farther north have higher concentrations of certain compounds (Figure 6 shows ΣPFCs). The loggerhead turtle with the highest concentrations of PFCs in the SC−FL sample set was described as an outlier in the analysis of bioaccumulation with turtle length (as seen in Figure 4). This 71.4-cm SCL female was captured at the northernmost location within the SC−FL sampling site (Figure 1) and may be strongly influencing the latitudinal gradient. However, when this turtle was excluded from the multiple regressions, all relationships between capture latitude and PFC concentrations remained significant ($p \leq 0.034$), except for PFNA ($p = 0.083$).

The causes of these geographical differences are unknown. Possible confounding relationships with water temperature are not likely, because water temperature measured at the time and location of capture was not significantly correlated with latitude ($r^2 = -0.162$, $p = 0.266$). One likely explanation for higher concentrations in the northern turtles is their location relative to potential sources of PFC contamination. Juvenile turtles on the summer feeding grounds in Core Sound, NC, show high site fidelity (*19*) and may therefore be indicators of local contamination. Turtles near Georgetown, SC, and in Core Sound, NC, had higher concentrations of PFOS, ΣPFCs, and other individual PFCs (not PFOA) compared to turtles captured offshore of GA and northeastern FL. The coastal regions of the northern sites, including NC, are primarily agricultural areas, but the rivers emptying into these locations, such as the Neuse River and the Pee Dee Rivers, drain relatively large watersheds including urban

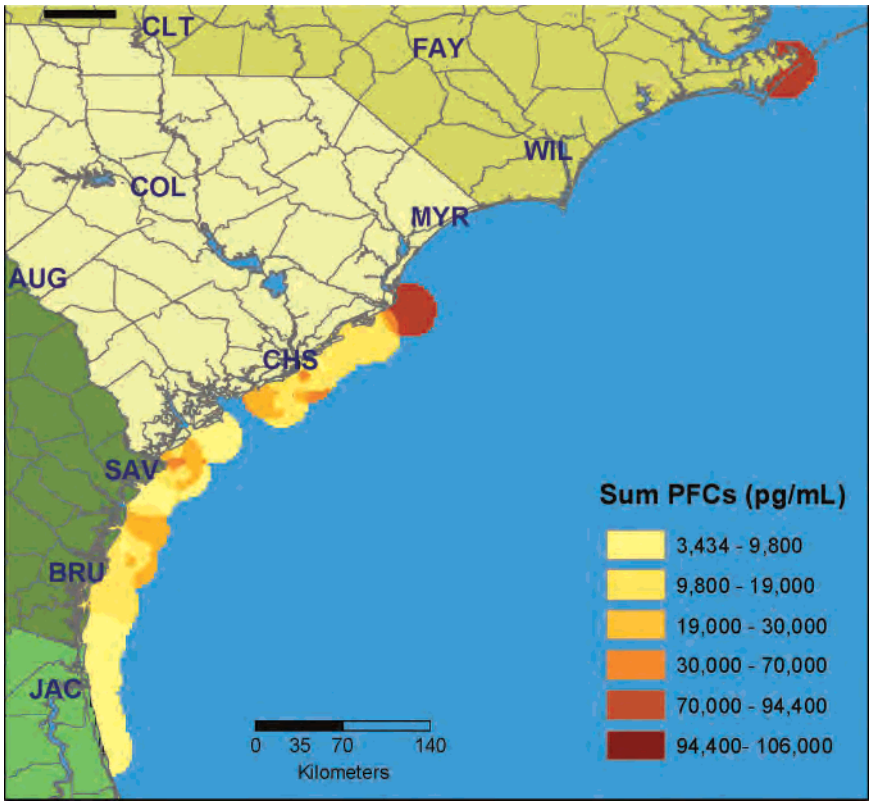


**FIGURE 6. Inverse distance-weighted interpolation map displaying ΣPFC concentrations measured in loggerhead sea turtle plasma based on their capture locations. Names of urban areas: JAC, Jacksonville; BRU, Brunswick; SAV, Savannah; AUG, Augusta; COL, Columbia; CHS, Charleston; MYR, Myrtle Beach; CLT, Charlotte; FAY, Fayetteville; WIL, Wilmington.**

centers and areas supporting many textile industries compared to smaller watersheds in GA and northeastern FL. It is possible that more local point sources are found in these larger northern watersheds. Another difference is that the northeastern FL portion of the study site is more influenced by the Gulf Stream than the northern sites, because the continental slope is much wider off of the GA and SC coast, and the NC site is protected by barrier islands. This results in more tropical, less productive waters that are rapidly turned over in offshore FL sites compared to slower water turnover rates and greater riverine influences in the northern sites. The watershed characteristics and turnover rate differences may result in larger PFC inputs into and slower export out of the northern sites. In addition, availability or selection of prey species might also vary with latitude, which could influence the bioaccumulation of PFCs in the turtles.

Actual point sources are unknown but could come from manufacturing, application, use, or disposal activities. Interestingly, other spatial studies indicate that the Carolinas may be more contaminated than other areas (*12*, *14*). People living in Charlotte, NC had the highest mean plasma PFOS concentrations compared to people living in 5 other U.S. cities (*14*). Bottlenose dolphins captured near Charleston, SC, had a higher mean plasma PFOS concentration compared to dolphins from two areas in FL and from Bermuda (*12*). Future studies should investigate the local sources in the Carolinas and analyze turtles captured from an expanded region, including farther north and south along the U.S. Atlantic coast and into the Gulf of Mexico to determine whether the more contaminated sites in the Carolinas are localized or part of a larger geographic gradient.

This study provides the first baseline concentrations for PFCs in sea turtle tissues and is among only a few to report PFC concentrations in reptiles (*4*, *6*). By taking advantage of the unique life history traits of sea turtles, interesting bioaccumulation patterns were noted and future studies should further investigate the maternal transfer of PFCs to sea turtle eggs and expand the geographical comparison. Because the turtles in this study were tagged and released, samples taken from them upon recapture could be useful in future studies to evaluate the effectiveness of 3M's phase-out of PFOS-based fluorochemical production in 2001. Moreover, ongoing studies addressing the health impacts of these compounds on sea turtles and other wildlife will begin to address the implications of environmental contamination with PFCs (*33*−*34*).

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## Supporting Information Available

Representative chromatogram showing perfluorinated compounds in loggerhead sea turtle plasma containing average concentrations. Multiple reaction monitoring ions and the maximum abundance value are provided in the upper right corner of each chromatogram. The values pointing at each peak from top to bottom indicate the retention time in minutes and the area under the curve. Note the low signal-to-noise ratio for PFHpA in this turtle, this compound was reported as <MDL in this sample. This material is available free of charge via the Internet at http://pubs.acs.org.

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