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Perfluorinated Alkyl Acids in plasma of American alligators (*Alligator mississippiensis*) from Florida and South Carolina

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Abstract:

This study aimed to quantitate fourteen perfluoroalkyl acids (PFAAs) in 125 adult American alligators at twelve sites across the southeastern US. Of those fourteen PFAAs, nine were detected in 65 % - 100 % of the samples: PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTriA, PFTA, PFHxS, and PFOS. Males (across all sites) showed significantly higher concentrations of four PFAAs: PFOS ($p = 0.01$), PFDA ($p = 0.0003$), PFUnA ($p = 0.021$), and PFTriA ($p = 0.021$). Concentrations of PFOS, PFHxS, and PFDA in plasma were significantly different among the sites in each sex. Alligators at Merritt Island National Wildlife Refuge and Kiawah Nature Conservancy both exhibited some of the highest PFOS concentrations (medians 99.5 ng/g and 55.8 ng/g respectively) in plasma measured to date in a crocodilian species. A number of positive correlations between PFAAs and snout-vent length (SVL) were observed in both sexes suggesting PFAA body burdens increase with increasing size. In addition, several significant correlations among PFAAs in alligator plasma may suggest conserved sources of PFAAs at each site throughout the greater study area. This study is the first to report PFAAs in American alligators, reveals potential PFAA hot spots in Florida and South Carolina, and provides and additional contaminant of concern when assessing anthropogenic impacts on ecosystem health.

Keywords: PFOS PFHxS alligator crocodilians plasma

INTRODUCTION

Despite being manufactured for over 50 years [1], it wasn't until 2000 that the class of chemicals known as perfluoroalkyl acids (PFAAs) entered the scientific spotlight as a major environmental contaminant of concern [2]. The two most commonly known PFAAs, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), were first produced by 3M in 1948 [1] and 1947, respectively, the latter of which was subsequently purchased by DuPont in 1951 [3]. A variety of new PFAAs have steadily been introduced to the market since the introduction of these first PFAAs. Structurally, PFAAs can widely vary, but as a whole, they typically consist of carbon chains of varying length (linear and branched isomers), an acid functional group, and hydrogen atoms substituted with fluorine atoms [4]. The carbon–fluorine bonds are the unique feature of PFAAs and provide chemical and thermal stability [5]. Two well-studied families of PFAAs are carboxylic acids and sulfonic acids [2, 6].

The usage of PFAAs has become widespread since the introduction of these chemicals in the 1940s, largely because they exhibit unique surfactant properties that make them attractive components for many consumer-related products, such as non-stick pans, water repellant surfaces, hair products, plastics, and lubricants [2], as well as firefighting products known as aqueous film-forming foams (AFFF) [7]. Active manufacturing and use of certain PFAAs, like PFOS and PFOA, have largely ceased owing to a voluntary phase-out by industry. Current production of fluorinated chemicals includes shorter chained carboxylic and sulfonic acid substitutes, like perfluorobutanesulfonic acid (PFBS) and perfluorobutyric acid (PFBA), respectively [8]. In addition, precursor chemicals that have a non-fluorinated structural component attached to a perfluorinated chain may be amenable to microbial or chemical transformation and have the potential to degrade into perfluorinated carboxylic and sulfonic acids over time [9].

The same properties that make PFAAs commercially valuable (e.g. the highly stable carbon fluorine bonds) also enable them to persist in the environment by resisting chemical, microbial, and photolytic degradation. However, unlike the more lipophilic environmental contaminants such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and brominated flame retardants (PBDEs) that are sequestered in adipose tissue, PFAAs accumulate in the blood and blood-rich organs, such as the liver [10, 11]. Conversely, like OCPs, PCBs, and PBDEs, PFAAs have also been shown to bioaccumulate and biomagnify in food webs [6]. Increasing PFAA chain length has been shown to correlate with an increasing ability to bioaccumulate [12], and the greatest PFAA concentrations detected in wildlife have been in species occupying high trophic positions [13]. Because PFAAs are bioaccumulative and often observed in higher concentrations in fish-eating marine species [13], humans who consume more fish in their diet may be at higher risk of PFAA exposure than those who consume less fish [14].

Animal studies reveal a wide range of PFAA-related effects that include alterations in liver physiology and serum cholesterol, as well as resulting hepatomegaly, wasting syndromes, neurotoxicity, immunotoxicity [15-17]. In addition, PFAAs have also been mentioned as possible obesogens due to their interaction with peroxisome proliferator activated receptors (PPAR) receptors [18]. However, although species-specific variations in PFAA excretion rates have been observed [2], the actual mechanism(s) of action of PFAA toxicity is not well understood.

Few reports exist on PFAA distribution and body burdens in North American wildlife, and studies of PFAAs in wild reptiles and amphibians have been limited almost exclusively to frogs and sea turtles [19]. Globally, only two studies have examined PFAAs in crocodilians [20, 21]. Because of their high trophic status, long life span, and high site fidelity, crocodilians are attractive study species for ecotoxicological investigations, particularly those involving exposure and accumulation of persistent environmental contaminants [23-25]. As such, studies examining PFAAs in crocodilians can provide insight into exposure and potential effects in focal species as well as identify potential hot spots of PFAA contamination.

In this study, we examined PFAA concentrations in plasma of wild American alligators (*Alligator mississippiensis*) from 12 sites in Florida and South Carolina (Figure 1). Because factors such as sex, body size, and location may influence PFAA concentrations in alligators, the relationships between PFAA body burdens and these parameters were also examined.

MATERIALS AND METHODS

Study Area

American alligator plasma samples (n = 125) were collected between 2012 and 2015, as part of multiple ongoing projects examining the biology and ecotoxicology of alligators in Florida and South Carolina [22-24]. In South Carolina, alligator blood samples were collected from the following sites (in order of North to South): Tom Yawkey Wildlife Center (YK, n = 10), Kiawah Island (KA, n =10), and Bear Island Wildlife Management Area (BI, n = 10) (Figure 1, Table S1). In Florida, samples were collected from the following sites (in order from North to South): Lochloosa Lake (LO, n = 10), Lake Woodruff (WO, n = 10), Lake Apopka (AP, n = 10), Merritt Island National Wildlife Refuge (MI, n = 15), St. John River (JR, n = 10), Lake Kissimmee (KS, n = 10), Lake Trafford (TR, n =10), Everglades Water Conservation Area 2A (2A, n = 10), and Everglades Water Conservation Area 3A (3A, n = 10) (Figure 1, Table S1).

Sample collection

Immediately following capture, a blood sample was collected from the post-occipital sinus of the spinal vein of each animal using a sterile needle and syringe [22-24]. Whole blood samples were then transferred to 8 mL lithium-heparin Vacutainer blood collection tubes (BD, Franklin Lakes, NJ), stored on ice in the field, and later centrifuged at 2500 rpm at 4 C for 10 min to obtain plasma, which was stored at -80 °C until analysis. Snout-vent length (SVL) was measured for each animal, and sex was determined by cloacal examination of the genitalia [25].

The National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM) 1958 Organic Contaminants in Fortified Human Serum was used as a control material during PFAA analysis. The freeze-dried human serum SRM 1958 was reconstituted with deioinized water according to the instructions on the Certificates of Analysis (www.nist.gov/srm/) and analyzed alongside collected alligator plasma.

Chemicals

Calibration solutions were created by combining two solutions produced by the NIST Reference Materials (RMs) 8446 Perfluorinated Carboxylic Acids and Perfluorooctane Sulfonamide in Methanol and RM 8447 Perfluorinated Sulfonic Acids in Methanol. Together, the solution contained 15 PFAAs as follows: PFBA, perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTriA), perfluorotetradecanoic acid (PFTA), PFBS, perfluorohexanesulfonic acid (PFHxS), PFOS, and perfluorooctanesulfonamide (PFOSA).

Internal standards (IS) were purchased from Cambridge Isotope Laboratories (Andover, MA), RTI International (Research Triangle Park, NC), and Wellington Laboratories (Guelph, Ontario) to create an internal standard (IS) mixture comprised of eleven isotopically labeled PFAAs, and they were as follows: $[^{13}C_4]$PFBA, $[^{13}C_2]$PFHxA, $[^{13}C_8]$PFOA, $[^{13}C_9]$PFNA, $[^{13}C_9]$PFDA, $[^{13}C_2]$PFUnA, $[^{13}C_2]$PFDoA, $[^{18}O_2]$PFBS, $[^{18}O_2]$PFHxS, $[^{13}C_4]$PFOS, and $[^{18}O_2]$PFOSA.

Sample preparation

Samples were extracted using a method previously described in 2011 by Reiner et al. [26]. Approximately 1 mL of each alligator plasma sample and SRM 1958 aliquots were thawed and gravimetrically weighed. All samples were then spiked with the IS mixture (approximately 600 µL) and gravimetrically weighed. After brief vortex-mixing and 90 min of equilibration, 4 mL of acetonitrile were used to extract the PFAAs from each sample. After sonication and centrifugation, the supernatant was removed from all samples. The collected supernatant was then solvent exchanged to methanol and further purified using an Envi-carb cartridge (Supelco, Bellefonte, PA). Resulting extracts were evaporated to 1 mL using nitrogen gas prior to being analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Samples were analyzed using an Agilent 1100 HPLC system (HPLC; Santa Clara, CA) coupled to an Applied Biosystems API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) with electrospray ionization in negative mode. Samples were examined by LC using an Agilent Zorbax Eclipse Plus C18 analytical column (2.1 mm x 150 mm x 5µm). A ramping LC solvent gradient was employed using methanol and de-ionized water both containing 20 mmol/L ammonium acetate [26]. Two multiple reaction monitoring (MRM) transitions for each PFAA were monitored to ensure no interferences with measurements, one MRM was employed for quantitation and the other one was used for confirmation [26].

Quality control

All alligator plasma samples were processed alongside quality control material NIST SRM 1958 and blanks to determine the accuracy and precision of the method. The PFAA levels of SRM 1958 processed during our extraction had to agree with previously established values reported on the Certificate of Analysis (CoA). In addition, compounds were considered to be above the reporting limit (RL) if the mass of an analyte in the sample was greater than the mean plus three standard deviations of all blanks.

Statistical Methods

All statistical analyses were performed using IBM SPSS statistic 22 (Armonk, NY: IBM Corp.). Statistical tests were performed for the compounds detected in greater than 75 % of the samples: PFNA, PFDA, PFUnA, PFDoA, PFTriA, PFTA, PFHxS, and PFOS. Unlike many environmental studies where PFOA is the second highest burden PFAA measured, PFOA was detected at much lower concentrations than many of the above PFAAs and was detected in only 65 % of the samples. With a full one third of PFOA measurements falling below the limit of detection (LOD), PFOA was excluded from statistical analysis along with the remaining chemicals (PFHpA, PFHxA, PFPeA, PFBS, and PFBA) that were detected in less than 2% of the samples. For those PFAAs included in statistical analysis, compounds less than the LOD were set equal to half the LOD prior to running the statistical tests [27].

Sex-based differences of PFAAs in Florida and South Carolina were investigated using univariate analysis of variance with log normally distributed concentration values, and a

Friedman's test was used for the PFAAs with non-normally distributed concentration values. Site was set as the nuisance factor, sex as the treatment, and PFAA burden as the dependent variable. These tests simulated a randomized block design for the collected data. Other parametric tests employed for data analysis of sex-based differences, on a site by site basis and analysis of site differences for PFAA levels, included a t-test and one-way ANOVA when data were normal or log-normal and Friedman rank test, Mann-Whitney U test and Kruskal Wallis test when data remained non-normal following log transformation. Pearson correlation and Spearman correlation were used when applicable for correlative measures.

RESULTS AND DISCUSSION

In this study, we collected a total of 125 plasma samples from alligators at multiple sites in Florida and South Carolina to examine PFAA concentrations in animals from different localities. Of the 15 PFAAs included in our analysis, all samples contained at least six. The following five PFAAs were detected in every plasma sample analyzed (in order of highest overall burden to lowest overall burden, among all sites): PFOS (median 11.2 ng/g, range 1.36–452 ng/g), PFUnA (median 1.58 ng/g, range 0.314–18.4 ng/g), PFDA (median 1.20 ng/g, range 0.169–15.1 ng/g), PFNA (median 0.528 ng/g, range 0.155–1.40 ng/g), and PFHxS (median 0.288 ng/g, range 0.057–23.3 ng/g) (Table 1 and Table S2). PFDoA, PFTriA, PFTA, and PFOA were also detected frequently in alligator plasma samples (over 96 %, 94 %, 75 %, and 65 %, respectively): PFDoA (median 0.363 ng/g, range <0.009–7.27 ng/g), PFTriA (median 0.416 ng/g, range <0.026–2.60 ng/g), PFTA (median 0.050 ng/g, range <0.008–1.38 ng/g), and PFOA (median 0.064 ng/g, range <0.008–0.412 ng/g) (Table 1 and Table S2). The nine PFAAs commonly measured over the LOD resulted in unique fingerprints for each site (Figure S1), which are discussed below. The shorter chain PFAAs (PFHpA, PFHxA, PFPeA, PFBS, and

PFBA) were detected infrequently (< 2 % of the samples) and therefore were not included in any statistical analysis.

Sex differences

Across all sites, male alligators exhibited significantly higher concentrations of several PFAAs in plasma compared to females as a group: PFOS (p = 0.01, Figure 2), PFDA (p = 0.0003, Figure S2), PFUnA (p = 0.021, Figure S2), and PFTriA (p = 0.021, Figure S2). However, at some sites, PFAA concentrations were significantly higher in females (e.g., PFOS at AP, PFUnA at KA).

In a population of captive Chinese alligators (*Alligator sinensis*), Wang et al. [21] found the highest PFAA concentrations in serum to be that of PFUnA rather than PFOS, the PFAA with the highest concentrations in our study. However, similar to our study, male Chinese alligators contained significantly higher concentrations of PFOS and PFUnA compared to females. Wang et al. [22] did not find a sex-based difference for PFDA in Chinese alligators. It is possible that sex-based differences observed for certain PFAAs in alligators is due to a differential clearance of these contaminants between males and females, as has been observed in rats [28], mice [29], and other mammals [30]. It is also possible females may offload PFAAs during oviposition, reducing their PFAA body burden compared to males at the same locality. This possibility is supported by studies reporting measurable concentrations of PFAAs in eggs of herring gulls (*Larus argentatus*) [31] and Nile crocodiles (*Crocodylus niloticus*) [20], confirming maternal transfer of PFAAs in oviparous species. Sex-specific differences in PFAA concentrations may also be the result of differential habitat use by adult males and females, a phenomenon common among crocodilians [32-35]. In such cases, differences are prey availability and contamination between and among habitats within a site could result in different PFAA exposures in males and females.

Because no sex-specific differences in PFOA, PFNA, PFHxS, PFDoA, and PFTA concentrations were observed among sampling localities in this study, sex-based differences were examined on a site-by-site basis (Table S3). Overall, only a few sites exhibited sex-based differences for these 5 PFAAs (Figure S3). At LO, male alligators had significantly higher PFNA ($p = 0.016$), PFTA ($p = 0.032$), and PFDoA ($p = 0.032$) plasma concentrations compared to females, and at MI males had significantly higher PFOA ($p = 0.047$) plasma concentrations than females. Interestingly, PFHxS was the only PFAA (of the five investigated on a site by site basis) for which females exhibited significantly higher plasma concentrations (YK, $p = 0.008$, TR, $p = 0.008$) when compared to males (Figure S3). It is important to note our examination of sex-based differences in PFAA concentrations may have been influenced by small samples sizes, as in almost all cases only five males and five females were sampled per site.

Site differences

Because sex-based differences in PFAA concentrations were observed among alligator plasma samples, site differences were determined separately for males and females. Of the nine detected PFAAs, all of them displayed at least some minor site differences. The PFAAs that displayed the most notable site differences (the most number of statistically significant groups between the 12 sites) were PFOS, PFDA, and PFHxS. Of those three, PFOS exhibited the greatest statistical difference across sites (Figure 3). This is likely due to the fact that PFOS is generally the most ubiquitous PFAA in the environment. When combining both sexes, concentrations of PFOS in alligator plasma ranged from 1.36 ng/g - 452 ng/g. For male alligators only, concentrations of PFOS in plasma ranged from 1.57 ng/g to 452 ng/g. PFOS concentrations were highest at MI (median 106 ng/g) and KA (median 56.4 ng/g). MI males exhibited significantly higher PFOS concentrations compared to all other sites with the exception of KA. In addition, the individual alligator with the highest overall

PFOS concentration measured in this study (452 ng/g plasma) was from MI. After MI, males from South Carolina (KA, YK, and BI) exhibited higher PFOS concentrations than Florida males, with the exception of WO. Some of the lowest PFOS concentrations observed in males in this study were measured at sites 2A, 3A, LO, and JR.

For female alligators, PFOS concentrations in plasma ranged from 1.36 ng/g - 206 ng/g. Similar to males, females at sites MI (median 85.5 ng/g) and KA (median 51.3 ng/g) exhibited significantly higher PFOS concentrations compared to the other sites examined, and the individual female with highest PFOS concentration was from MI (206 ng/g plasma). After MI and KA, females from the two other South Carolina sites (YK and BI) exhibited higher PFOS concentrations than males from Florida, with the exception of WO and AP. Some of the lowest PFOS concentrations observed in females in this study were measured at sites 2A, 3A, LO, JR, and TR.

The concentrations of PFHxS detected in alligator plasma in this study exhibited a similar trend to PFOS across sites, but on a reduced scale (Table S4). Male and female PFHxS plasma concentrations ranged from 0.0566 ng/g – 23.3 ng/g throughout the sampling sites the entire southeast. For males, PFHxS plasma concentrations ranged from 0.057 ng/g – 23.3 ng/g. Males from MI (median 3.95 ng/g) had significantly higher PFHxS concentrations than any other site examined, and the individual male with highest PFHxS concentration was from site MI (23.3

ng/g). Males from KA and KS followed closely, but were still statistically grouped with other sites (AP, WO, and BI). The lowest PFHxS concentrations in males were typically measured at sites 2A, 3A, LO, and TR. For female alligators, PFHxS concentrations in plasma ranged from 0.069 ng/g – 10.0 ng/g. Like males, MI females exhibited significantly higher PFHxS concentrations than all other sites. Females at KA and KS had the next highest concentrations but were still statistically grouped with other sites (AP, WO, and YK). The lowest PFHxS concentrations in females were typically observed at sites 2A, 3A, and LO.

PFDA had a unique signature across the sampling sites, one that varied from the patterns observed for plasma PFOS and PFHxS concentrations (Table S4). PFDA plasma concentrations ranged from 0.169 ng/g - 15.1 ng/g for all sites examined. For male alligators, PFDA concentrations ranged from 0.498 ng/g – 15.1 ng/g. KA males had significantly higher PFDA concentrations overall (median 6.21 ng/g) compared to all sites, with the exception of YK (median 6.20 ng/g). YK males exhibited the next highest PFDA concentrations, but these were not significantly different from those detected in WO males (median 2.02 ng/g). Males at many of the remaining sites had similarly low concentrations of PFDA. Overall, LO males (median 0.792 ng/g) had some of the lowest PFDA concentrations of all the sampling sites. For female alligators, PFDA plasma concentrations ranged from 1.69 ng/g – 14.3 ng/g. The two sites with the highest (statistically significant) PFDA concentrations in females were also in South Carolina: KA (median 6.32 ng/g) and YK (median 5.55 ng/g). PFDA concentrations at BI (median 1.18 ng/g) and WO (median1.84) followed closely behind, but were not significantly different from the other sites sampled. Like males, LO females (median 0.501 ng/g) had some of the lowest PFDA concentrations across all sites.

Overall, male and female alligators from both MI and KA exhibited some of the highest PFOS concentrations measured to date in a crocodilian species (median PFOS concentrations in plasma: MI males = 106 ng/g, MI females = 85.5 ng/g. KA males = 56.4 ng/g, KA females = 51.3 ng/g). In comparison, the mean PFOS concentration in serum from captive Chinese Alligators was 28.7 ng/mL (28.0 ng/g) [21]. In other reptiles, Kemp's ridley sea turtles (*Lepidochelys kempii*) and loggerhead sea turtles (*Caretta caretta*) along the coast of South Carolina, Georgia, and Florida exhibited median PFOS plasma concentrations of 41900 pg/ml (40.9 ng/g) and 5450 pg/ml (53.2 ng/g), respectively [27]. The high concentrations of PFOS and PFHxS detected in male and female alligators at MI may be related to the aeronautic facilities located in and around MI that comprise up a large part of Florida's Kenndey Space Center. The use of AFFF is not uncommon at Kennedy Space Center, and may contribute to PFAAs in the surrounding environment. Historically, AFFF have been shown to contain PFAAs, such as PFOS and PFHxS, as well as a number of other propriety PFAA mixtures [7] and can be resistant to remediation [9]. PFAAs have been measured in firefighters [36], wildlife [6], and downstream of their use [37]. Potential sources of PFOS and PFHxS at KA are more speculative. In addition, it should be noted with the exclusion of MI, alligators from the South Carolina sites (BI, YK, and KA) had some of the highest PFOS concentrations compared to the Florida sites. In Florida, WO exhibited mid to high concentrations of PFOS, PFHxS, and PFDA compared to other sampled sites. For many years, WO has been used as a reference site for multiple studies on alligator ecotoxicology due to its relatively low concentrations of organochlorine contaminants, such as DDT, its metabolites, and other OCPs [38]. The results of the present study obviously indicate WO would not be a suitable reference site for future studies involving PFAAs. In contrast to WO, sites 2A and 3A, which are located in the Everglades, exhibited some of the lowest concentrations of PFOS and PFHxS measured in Florida. Interestingly, while PFAA concentrations appear to be relatively low, adult alligators at these sites have been reported to contain some of the highest mercury concentrations in Florida and throughout the range of the species [24, 39, 40].

Correlations

For all alligators included in the study, SVL was uniform across sites for males and nearly uniform across sites for females (Figure S4). Thus, data from all sites were combined within each sex to investigate relationships between PFAA burden and alligator SVL. Correlations comparing both male SVL to PFAAs and female SVL to PFAAs resulted in a number of significant positive correlations (Table 2). Overall, females exhibited higher correlation coefficients between PFAA concentration and SVL when compared to the males. The highest correlation coefficients for females were with PFTriA, which explained 57.0 % of the variation, followed closely by PFOS, which explained 55.1 % of the variation. In contrast, the highest correlation coefficients for male SVL and PFAA concentration was PFUnA, which explained 35.5 % of the variation, followed closely by PFOS, which explained 33.1 % of the variation. Collectively, these data suggest concentrations of some PFAAs in adult American alligators increase with increasing body size in both males and females. Conversely, Wang *et al.* [21] found that PFAA (PFUnA, PFDA, and PFNA) concentration decreased with increasing body size (total length). These observed differences between American and Chinese alligators may be the result of interspecific differences in food consumption, growth rates, and body size [41] as well as in toxicodynamics and toxicokinetics of PFAAs. In addition, differences in diet and numerous environmental variables between wild (this study) and captive [22] alligators may influence growth and body burdens of PFAAs. Finally, differences in sample types (plasma vs serum) between the two studies may have had some effect on PFAA-body size relationships.

With all sites combined for each sex, significant correlations were observed between different PFAAs measured in plasma, suggesting somewhat similar sources of PFAA contamination across the sampling localities. The varying levels of PFAA contamination from site to site are likely due to varying distances from these potential PFAA sources. Some correlative relationships between the PFAAs were stronger than others (Table 3). Of all the PFAAs, correlations between PFUnA and PFDoA for male ($p < 0.01$, $r = 0.920$) and female ($p < 0.01$, $r = 0.938$) alligators across the sites were the most highly significant relationships observed in this study.

CONCLUSIONS

This study is the first to quantitate PFAA concentrations in American alligators and one of the few studies to quantitate PFAAs in crocodilians [20, 21]. All alligator samples (n = 125) contained at least six quantifiable PFAAs: PFOS (median 11.2 ng/g, range 1.36–452 ng/g), PFUnA (median 1.58 ng/g, range 0.314–18.4 ng/g), PFDA (median 1.20 ng/g, range 0.169–15.1 ng/g), PFNA (median 0.528 ng/g, range 0.155–1.40 ng/g), and PFHxS (median 0.288 ng/g, range 0.057–23.3 ng/g). Our findings support sex-based differences in PFOS and PFUnA concentrations previously observed in captive Chinese alligators [21], while demonstrating opposite relationships between PFAA concentration and body size exist for American (wild) and Chinese (captive) alligators A high number of significant PFAA to PFAA correlations suggest common point sources throughout the samplimg sites in Florida and South Carolina. This study also reveals potential hot spots for various PFAAs (e.g., PFOS at KA and MI) that warrant further investigation. and provides another contaminant of concern to be combined with organochlorines, metals, and others when assessing overall anthropogenic impacts on ecosystem health.

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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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FIGURE LEGENDS

Figure 1. Map showing the twelve sites from which American alligators (*Alligator mississippiensis*) were sampled in this study (n = 125) during the years 2012 – 2015. Collection sites are listed in decreasing latitude.

Figure 2. Mean (±SD) PFOS concentrations (log; ng/g) in male and female American alligators (*Alligator mississippiensis*) sampled at multiple sites in Florida and South Carolina. Samples are listed from left to right in decreasing latitude. Refer to figure 1 for site abbreviations.

Figure 3. Mean (±SD) PFOS concentrations (log; ng/g) in **(A)** male and **(B)** female American alligator (*Alligator mississippiensis*) plasma from multiple sites in Florida and South Carolina. Letters above bars represent statistically significant differences between groups ($p < 0.05$).Samples are listed from left to right in decreasing latitude. Refer to figure 1 for site abbreviations.

Table 1. Alligator perfluoroalkyl acid (PFAA) plasma concentrations (ng/g wet mass) at 12 sites from Florida and South Carolina: Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTriA), perfluorotetradecanoic acid (PFTA), perfluorohexanesulfonic acid (PFHxS), and perfluorooctane sulfonate (PFOS).

	Lake Apopka (AP) n = 10			**Bear Island (BI)** n = 10			**Kiawah Island (KA)** n = 10			**Lake Kissimmee (KS)** n = 10			**Lochloosa Lake (LO)** n = 10			**Merrit Island (MI)** n = 15		
	Range	Median	n > RL[a]	Range	Median	n > RL[a]	Range	Median	n > RL[a]	Range	Median	n > RL[a]	Range	Median	n > RL[a]	Range	Median	n > RL[a]
PFOA	<0.096[b]-0.152	0.126	7	<0.008[b]-0.193	< 0.100	3	0.028-0.298	0.126	10	<0.008[b]-0.142	0.104	9	<0.008[b]-0.132	0.071	9	<0.096[b]-0.412	0.155	7
PFNA	0.251-1.40	0.648	10	0.155-1.14	0.472	10	0.446-1.38	1.19	10	0.275-1.18	0.642	10	0.328-1.19	0.676	10	0.298-1.10	0.611	15
PFDA	0.169-2.44	1.12	10	0.998-3.21	1.57	10	3.72-13.6	6.26	10	0.417-3.15	1.26	10	0.238-1.00	0.615	10	0.395-3.50	1.02	15
PFUnA	0.614-3.39	1.65	10	1.05-5.02	2.32	10	1.87-7.53	3.93	10	0.314-2.47	1.03	10	0.580-1.56	1.03	10	0.844-5.45	1.82	15
PFDoA	<0.157[b]-0.831	0.315	9	0.231-1.88	0.559	10	1.32-7.27	3.05	10	<0.009[b]-0.382	0.147	9	0.105-0.309	0.182	10	<0.543[b]-1.07	0.418	14
PFTriA	0.189-1.00	0.450	10	<0.070[b]-1.83	0.674	9	0.420-2.60	0.919	10	0.122-0.677	0.251	10	0.181-0.580	0.309	10	<0.026[b]-1.42	0.654	14
PFTA	<0.080[b]-0.194	0.049	7	<0.081[b]-0.733	0.095	7	0.198-1.38	0.476	10	<0.009[b]-0.104	0.025	9	<0.008[b]-0.060	0.018	7	<0.080[b]-0.257	0.076	6
PFHxS	0.166-0.449	0.332	10	0.077-0.824	0.304	10	0.313-1.86	0.620	10	0.338-1.50	0.505	10	0.069-0.201	0.093	10	0.684-23.3	3.83	15
PFOS	1.98-15.8	11.4	10	10.0-44.9	19.5	10	38.4-98.2	55.8	10	6.51-25.1	12.2	10	2.19-6.16	4.21	10	38.6-452	99.5	15

	St. Johns River (JR) n = 10			**Lake Trafford (TR)** n = 10			**WCA-2A (2A)** n = 10			**WCA-3A (3A)** n = 10			**Lake Woodruff (WO)** n = 10			**Yawkey (YK)** n = 10		
	Range	Median	n > RL[a]	Range	Median	n > RL[a]	Range	Median	n > RL[a]	Range	Median	n > RL[a]	Range	Median	n > RL[a]	Range	Median	n > RL[a]
PFOA	0.010-0.160	0.080	10	0.021-0.117	0.091	10	<0.008[b]-0.077	0.036	2	<0.008[b]-0.042	0.033	6	<0.097[b]-0.184	0.062	5	<0.008[b]-0.193	0.050	4
PFNA	0.250-1.04	0.471	10	0.239-0.936	0.484	10	0.189-0.382	0.234	10	0.172-0.388	0.301	10	0.282-1.34	0.578	10	0.272-1.32	0.620	10
PFDA	0.492-1.72	1.17	10	0.275-2.05	0.885	10	0.641-2.26	0.900	10	0.406-1.46	0.912	10	0.350-5.06	2.01	10	2.27-15.1	5.88	10
PFUnA	0.655-2.20	1.28	10	0.463-2.19	0.953	10	0.958-3.15	1.43	10	0.719-2.48	1.45	10	0.633-3.33	1.43	10	1.89-18.4	6.25	10
PFDoA	0.156-0.591	0.362	10	0.073-0.737	0.210	10	0.277-0.949	0.392	10	0.172-0.631	0.371	10	<0.166[b]-0.810	0.317	9	0.362-3.45	1.01	10
PFTriA	0.173-0.739	0.267	10	0.111-0.528	0.304	10	0.232-0.702	0.370	10	0.162-0.594	0.280	10	<0.070[b]-0.854	0.259	8	<0.070[b]-1.85	0.646	8
PFTA	<0.008[b]-0.131	0.022	8	<0.008[b]-0.096	0.039	9	0.031-0.188	0.109	10	0.011-0.148	0.042	10	<0.008[b]-0.146	0.029	4	<0.082[b]-0.774	0.241	7
PFHxS	0.100-0.308	0.166	10	0.071-0.320	0.119	10	0.080-0.172	0.112	10	0.057-0.303	0.105	10	0.130-0.623	0.445	10	0.099-0.566	0.353	10
PFOS	3.41-10.2	7.13	10	4.21-14.3	7.82	10	1.36-6.23	2.65	10	1.57-4.71	3.81	10	5.89-41.2	16.0	10	4.50-57.0	20.2	10

[a]n > RL indicates the number of samples above the reporting limit (RL)

[b]Values were calculated with half the RL substituted for nondetects as described in the methods section, but values shown as "<" a specified number describe the actual RL

NA = Not applicable

Table 2. Correlations between plasma PFAA concentrations and snout-vent length (SVL) for American alligators (*Alligator mississippiensis*) sampled in Florida and South Carolina (n_{male} = 65, n_{female} = 60). Refer to table 1 for PFAA abbreviations. Values were calculated using log normal concentrations. **Bold** indicates significant correlation coefficients.

SVL	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTA	PFTriA	PFHxS	PFOS
Male	0.072	**0.252**[a]	0.206	**0.355**[b]	**0.279**[a]	0.209	**0.273**[a]	**0.273**[a]	**0.331**[b]
Female	0.133	**0.261**[a]	**0.443**[b]	**0.489**[b]	**0.469**[b]	**0.468**[b]	**0.570**[b]	**0.412**[b]	**0.551**[b]

[a] Correlation is significant at the 0.05 level (2-tailed).

[b] Correlation is significant at the 0.01 level (2-tailed).

Table 3. Correlations between concentrations of various PFAAs in plasma of American alligators (*Alligator mississippiensis*) sampled in Florida and South Carolina (n_{male} = 65, n_{female} = 60). Refer to table 1 for PFAA abbreviations. Values were calculated using log normal concentrations. **Bold** indicates significant correlation coefficients.

Male	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFHxS	PFOS
PFOA	-	**0.615**[b]	0.226	0.092	0.036	0.152	0.181	0.260	**0.386**[b]
PFNA		-	**0.550**[b]	**0.322**[a]	0.144	**0.313**[a]	**0.273**[b]	**0.339**[b]	**0.541**[b]
PFDA			-	**0.840**[b]	**0.743**[b]	**0.439**[b]	**0.654**[b]	**0.307**[a]	**0.550**[b]
PFUnA				-	**0.920**[b]	**0.783**[b]	**0.826**[b]	**0.445**[b]	**0.654**[b]
PFDoA					-	**0.751**[b]	**0.846**[b]	**0.316**[a]	**0.528**[b]
PFTriA						-	**0.770**[b]	**0.395**[b]	**0.489**[b]
PFTA							-	0.238	**0.399**[b]
PFHxS								-	**0.827**[b]
PFOS									-

Female	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFHxS	PFOS
PFOA	-	**0.648**[b]	**0.332**[a]	0.186	0.098	0.064	-0.003	**0.441**[b]	**0.440**[b]
PFNA		-	**0.585**[b]	**0.444**[b]	**0.365**[b]	**0.339**[b]	0.196	**0.387**[b]	**0.538**[b]
PFDA			-	**0.890**[b]	**0.827**[b]	**0.529**[b]	**0.560**[b]	**0.337**[b]	**0.595**[b]
PFUnA				-	**0.938**[b]	**0.684**[b]	**0.578**[b]	**0.331**[a]	**0.691**[b]
PFDoA					-	**0.763**[b]	**0.708**[b]	0.226	**0.635**[b]
PFTriA						-	**0.713**[b]	0.190	**0.598**[b]
PFTA							-	0.130	**0.454**[b]
PFHxS								-	**0.654**[b]
PFOS									-

[a] Correlation is significant at the 0.05 level (2-tailed).

[b] Correlation is significant at the 0.01 level (2-tailed).

Figure 1.

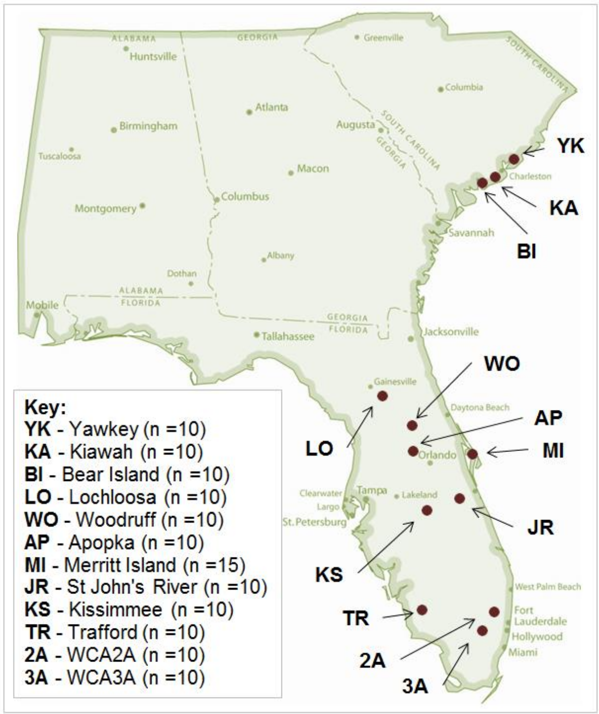
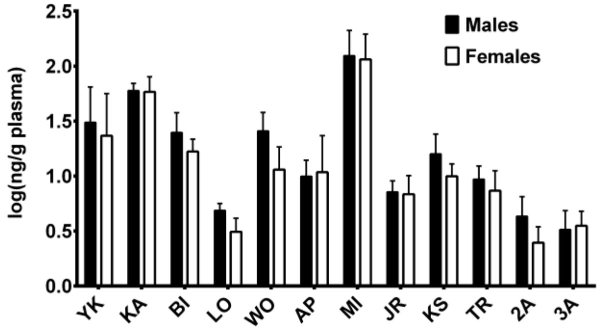

YK
KA
BI
WO
AP
MI
LO
JR
KS
TR
2A
3A
Key:
YK - Yawkey (n =10)
KA - Kiawah (n =10)
BI - Bear Island (n =10)
LO - Lochloosa (n =10)
WO - Woodruff (n =10)
AP - Apopka (n =10)
MI - Merritt Island (n =15)
JR - St John's River (n =10)
KS - Kissimmee (n =10)
TR - Trafford (n =10)
2A - WCA2A (n =10)
3A - WCA3A (n =10)

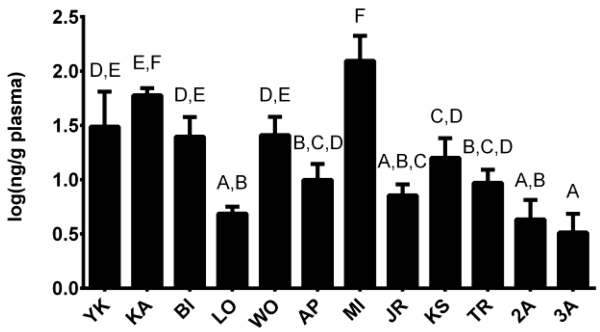
Figure 2.



Males
Females
log(ng/g plasma)
2.5
2.0
1.5
1.0
0.5
0.0
YK
KA
BI
LO
WO
AP
MI
JR
KS
TR
2A
3A

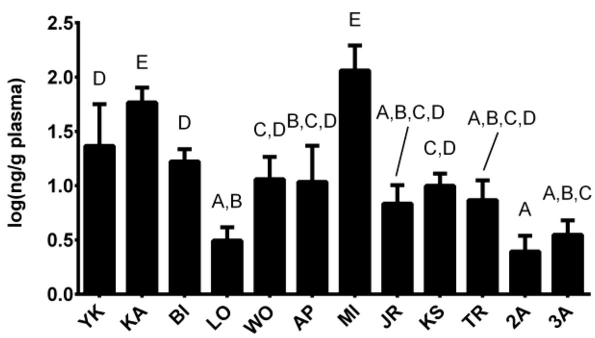
Figure 3.

A



log(ng/g plasma)
2.5
2.0
1.5
1.0
0.5
0.0
D,E
E,F
D,E
A,B
D,E
B,C,D
F
A,B,C
C,D
B,C,D
A,B
A
YK
KA
BI
LO
WO
AP
MI
JR
KS
TR
2A
3A

B



log(ng/g plasma)
2.5
2.0
1.5
1.0
0.5
0.0
D
E
D
A,B
C,D
B,C,D
E
A,B,C,D
C,D
A,B,C,D
A
A,B,C
YK
KA
BI
LO
WO
AP
MI
JR
KS
TR
2A
3A

SUPPLEMENTAL INFORMATION

Table S1. American alligator plasma sampling site descriptions

Sampling Location	Abbreviation	State	Coastal vs Inland	^{0}N	^{0}W	Year(s)	n
Tom Yawkey Wildlife Center	YK	SC	Costal	33.107	79.132	2014	10
Kiawah Island	KA	SC	Coastal	32.363	80.045	2015	10
Bear Island Wildlife Management Area	BI	SC	Coastal	32.364	80.264	2014	10
Lochloosa Lake	LO	FL	Inland	29.496	82.152	2012	10
Lake Woodruff	WO	FL	Inland	29.107	81.404	2014	10
Lake Apopka	AP	FL	Inland	28.614	81.634	2014	10
Merritt Island National Wildlife Refuge	MI	FL	Coastal	28.523	80.682	2011-2014	15
St. Johns River	JR	FL	Inland	28.196	80.820	2012	10
Lake Kissimmee	KS	FL	Inland	27.905	81.222	2012	10
Lake Trafford	TR	FL	Inland	26.436	81.499	2012	10
Water Conservation Area 2A	2A	FL	Inland	26.319	80.523	2012	10
Water Conservation Area 3A	3A	FL	Inland	26.215	80.689	2012	10

Table S2. PFAA concentrations (ng/g) in American alligator (*Alligator mississippiensis*) plasma from multiple sites in Florida and South Carolina. Values shown as “<” a specified number describe the actual reporting limit. SVL = snout-vent lengthRefer to table 1 and figure 1 for chemical and site abbreviations, respectively. NA = Not available.

Collection Site	Sex	SVL (cm)	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFHxS	PFOS
YK	F	111.0	<0.099	0.398	4.13	5.72	0.552	<0.070	<0.082	0.356	10.4
YK	F	120.0	<0.103	0.439	2.27	3.57	0.525	<0.080	<0.086	0.566	4.50
YK	F	131.0	<0.100	0.894	14.3	18.4	2.24	1.04	0.329	0.547	31.6
YK	F	135.0	<0.099	0.735	5.55	8.41	1.06	0.624	<0.082	0.510	12.8
YK	F	144.8	<0.099	0.272	9.63	12.6	1.79	1.37	0.406	0.470	57.0
YK	M	106.7	0.193	1.17	6.20	5.03	0.962	0.668	0.148	0.131	24.6
YK	M	165.1	0.014	1.24	7.62	6.77	1.57	1.15	0.241	0.288	50.8
YK	M	170.2	0.131	1.32	15.1	11.9	3.45	1.85	0.774	0.351	55.2
YK	M	171.5	<0.008	0.286	2.89	2.93	0.672	0.485	0.118	0.099	15.9
YK	M	NA	0.008	0.506	2.61	1.89	0.362	0.345	0.076	0.107	7.57
KA	F	109.2	0.028	0.952	6.59	6.11	6.98	2.60	1.38	0.313	57.2
KA	F	119.4	0.105	1.27	6.39	4.05	3.02	0.821	0.432	0.351	38.4
KA	F	123.8	0.055	1.14	4.03	3.41	2.91	1.65	0.606	0.374	51.3
KA	F	137.4	0.242	1.36	3.72	1.87	1.32	0.420	0.284	1.23	46.9
KA	F	141.0	0.129	1.37	6.32	4.06	3.09	1.16	0.689	1.86	98.2
KA	M	91.0	0.069	0.446	5.12	3.26	2.51	0.671	0.401	0.499	56.4
KA	M	112.4	0.298	0.824	6.52	3.98	3.85	0.857	0.657	1.52	65.0
KA	M	132.0	0.218	1.38	13.6	7.53	7.27	1.04	0.198	0.825	74.5
KA	M	143.0	0.209	1.02	6.13	3.89	3.26	0.980	0.520	0.742	55.2
KA	M	168.0	0.124	1.24	6.21	3.54	1.91	0.550	0.243	0.451	48.1
BI	F	108.0	<0.213	0.690	1.18	2.46	0.454	0.674	<0.177	0.824	17.2
BI	F	109.0	0.193	1.14	2.01	2.05	0.470	0.414	0.101	0.220	16.1
BI	F	134.0	<0.098	0.359	0.998	2.17	0.522	1.02	<0.082	0.411	18.2
BI	F	136.6	<0.008	0.155	1.75	2.05	0.653	1.01	0.246	0.101	22.0
BI	F	162.2	<0.008	0.462	1.18	1.06	0.259	0.352	0.083	0.077	10.0
BI	M	118.0	0.105	0.556	1.20	1.05	0.231	0.244	0.039	0.142	12.2
BI	M	128.0	0.089	0.781	3.21	3.22	0.832	0.839	0.202	0.148	24.0
BI	M	128.0	<0.097	0.483	2.37	3.74	0.596	<0.070	<0.081	0.388	20.8
BI	M	157.6	<0.099	0.371	1.39	2.76	0.795	0.428	0.193	0.440	22.5
BI	M	168.0	<0.100	0.339	2.38	5.02	1.88	1.83	0.733	0.510	44.9
AP	F	103.8	0.128	0.911	2.03	3.14	0.831	1.00	0.194	0.177	15.8
AP	F	111.0	0.143	1.40	2.44	3.39	0.564	0.547	0.050	0.373	13.3
AP	F	114.5	<0.096	0.251	0.169	0.614	<0.157	0.189	<0.080	0.379	1.98
AP	F	130.5	0.121	1.01	1.15	1.61	0.299	0.441	0.055	0.291	13.2
AP	F	144.0	0.152	0.492	0.839	1.25	0.260	0.390	0.047	0.166	9.93
AP	M	122.9	<0.098	0.471	0.691	1.68	0.360	0.615	<0.082	0.449	7.26
AP	M	136.0	<0.098	0.296	0.520	1.10	0.207	0.464	<0.082	0.388	6.31
AP	M	146.0	0.124	0.512	1.09	1.74	0.300	0.416	0.044	0.256	8.14
AP	M	162.0	0.140	0.784	1.90	2.38	0.528	0.559	0.091	0.190	12.9
AP	M	185.0	0.147	1.08	1.90	1.36	0.330	0.317	0.052	0.435	15.1
LO	F	76.0	0.079	0.577	0.543	0.848	0.114	0.213	<0.009	0.069	2.43
LO	F	76.1	<0.008	0.328	0.238	0.580	0.105	0.181	<0.008	0.093	2.19
LO	F	94.0	0.095	0.720	0.596	0.930	0.120	0.250	<0.008	0.092	2.96
LO	F	105.5	0.010	0.561	0.501	1.03	0.218	0.312	0.012	0.104	4.98
LO	F	144.0	0.008	0.416	0.425	0.858	0.158	0.352	0.033	0.079	3.02
LO	M	95.8	0.132	0.956	0.708	1.04	0.161	0.284	0.019	0.078	4.18
LO	M	108.8	0.064	0.891	0.634	1.10	0.202	0.306	0.017	0.105	4.24
LO	M	128.4	0.125	1.19	0.996	1.56	0.277	0.471	0.052	0.121	5.18
LO	M	159.0	0.073	0.632	0.825	1.33	0.273	0.580	0.060	0.093	4.63
LO	M	186.0	0.069	1.15	0.792	1.49	0.309	0.487	0.060	0.201	6.16
WO	F	99.0	0.105	0.799	2.16	1.49	0.342	0.192	<0.009	0.234	12.1
WO	F	102.0	<0.101	0.282	0.350	0.633	<0.166	0.102	<0.084	0.457	5.89
WO	F	103.5	0.074	1.08	3.06	1.98	0.386	0.373	0.049	0.138	19.8
WO	F	106.4	<0.101	0.313	0.717	1.20	0.218	<0.070	<0.084	0.471	6.04
WO	F	113.0	0.184	1.05	1.84	1.24	0.291	0.272	0.037	0.433	13.5
WO	M	58.5	<0.100	0.505	2.03	2.01	0.386	0.854	<0.083	0.530	22.0
WO	M	135.0	<0.097	0.482	1.38	1.14	0.224	<0.070	<0.080	0.546	15.8
WO	M	157.3	0.152	1.34	5.06	3.33	0.810	0.697	0.146	0.388	41.2
WO	M	170.0	<0.096	0.603	2.22	2.34	0.465	0.315	<0.080	0.623	33.7
WO	M	171.0	0.087	0.554	1.99	1.36	0.265	0.246	0.020	0.130	16.2

Table S2. (continued)

Collection Site	Sex	SVL (cm)	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFHxS	PFOS
MI	F	121.0	<0.098	0.410	0.753	1.39	0.203	0.915	<0.082	1.72	43.0
MI	F	123.0	<0.096	0.298	0.395	0.844	0.190	0.961	<0.080	3.01	178
MI	F	129.0	<0.096	0.611	1.02	1.87	0.276	0.608	<0.080	2.32	62.0
MI	F	NA	0.115	1.10	2.08	1.92	0.717	0.413	0.152	10.0	206
MI	F	NA	0.037	0.577	0.816	1.02	0.191	0.197	0.036	3.98	85.5
MI	M	135.0	<0.332	0.895	1.66	2.88	<0.543	<0.026	<0.275	2.56	99.5
MI	M	135.0	0.235	0.590	0.938	2.64	0.393	0.948	<0.088	1.46	43.2
MI	M	136.0	<0.096	0.528	1.20	2.83	0.400	0.674	<0.080	1.50	38.6
MI	M	143.0	<0.097	0.855	3.49	5.45	1.07	0.684	0.257	7.33	452
MI	M	144.0	<0.098	1.10	1.80	3.31	0.607	0.564	0.098	3.83	118
MI	M	152.0	0.306	0.653	0.507	1.04	0.209	1.05	<0.081	4.07	52.6
MI	M	154.0	0.144	0.483	0.944	1.38	0.275	0.635	<0.082	4.87	113
MI	M	154.0	0.412	0.740	1.33	1.69	0.445	1.42	0.219	4.20	115
MI	M	163.0	0.199	0.798	1.40	1.82	0.440	0.474	0.165	23.3	172
MI	M	181.0	<0.133	0.347	0.725	1.75	0.435	0.425	<0.110	0.684	38.8
JR	F	86.0	0.160	1.04	1.72	2.20	0.432	0.205	<0.008	0.124	9.31
JR	F	88.0	0.017	0.369	0.492	0.655	0.156	0.173	0.013	0.308	3.41
JR	F	96.0	0.019	0.368	1.20	1.03	0.226	0.203	0.017	0.221	9.23
JR	F	126.0	0.083	0.447	0.843	0.903	0.234	0.232	0.023	0.165	4.88
JR	F	135.6	0.010	0.494	0.870	1.31	0.591	0.739	0.131	0.219	7.33
JR	M	117.5	0.109	0.692	1.14	1.25	0.271	0.321	0.045	0.177	5.25
JR	M	126.0	0.082	0.356	1.23	1.31	0.399	0.406	0.081	0.105	7.82
JR	M	137.0	0.011	0.603	1.45	1.87	0.441	0.359	0.021	0.166	6.92
JR	M	146.0	0.079	0.540	1.51	1.63	0.438	0.447	0.081	0.108	10.2
JR	M	168.2	0.082	0.250	0.740	0.994	0.326	0.227	<0.008	0.100	5.76
KS	F	90.0	0.113	0.860	1.17	0.841	0.148	0.252	0.028	0.476	7.88
KS	F	90.4	0.105	0.275	0.417	0.314	<0.009	0.122	<0.009	1.50	6.51
KS	F	106.2	0.115	1.18	2.08	1.73	0.346	0.455	0.085	1.17	13.1
KS	F	115.1	0.102	0.542	1.12	0.909	0.114	0.229	0.017	0.719	11.2
KS	F	124.0	0.125	0.591	1.32	1.08	0.138	0.229	0.015	0.612	11.3
KS	M	96.0	0.079	0.693	0.866	0.760	0.131	0.225	0.022	0.338	7.48
KS	M	109.2	0.017	0.463	1.19	0.990	0.147	0.250	0.019	0.493	13.3
KS	M	142.5	0.093	0.507	1.53	1.34	0.165	0.295	0.038	0.485	13.3
KS	M	160.0	<0.008	0.928	3.15	2.47	0.382	0.677	0.104	0.486	25.1
KS	M	167.0	0.142	0.836	2.28	1.72	0.301	0.485	0.079	0.517	20.3
TR	F	50.6	0.117	0.555	0.640	0.694	0.148	0.159	0.013	0.176	7.41
TR	F	70.5	0.021	0.239	0.275	0.463	0.112	0.111	<0.008	0.127	4.21
TR	F	86.9	0.094	0.284	0.424	0.533	0.073	0.169	0.014	0.123	5.30
TR	F	93.0	0.067	0.438	0.488	0.619	0.125	0.152	0.018	0.320	5.61
TR	F	105.0	0.066	0.458	1.46	2.15	0.737	0.509	0.096	0.123	14.3
TR	M	99.0	0.099	0.510	1.13	1.15	0.257	0.331	0.036	0.112	13.4
TR	M	109.0	0.087	0.392	0.617	0.753	0.162	0.278	0.035	0.088	5.96
TR	M	134.0	0.099	0.803	1.44	1.44	0.296	0.435	0.086	0.091	8.25
TR	M	139.9	0.097	0.646	1.66	1.56	0.342	0.440	0.074	0.071	8.23
TR	M	142.0	0.022	0.936	2.05	2.19	0.522	0.528	0.055	0.116	10.9
2A	F	62.0	<0.008	0.251	0.887	1.29	0.281	0.232	0.056	0.121	3.74
2A	F	88.0	0.077	0.237	0.708	1.09	0.409	0.398	0.140	0.080	2.34
2A	F	91.4	<0.072	0.189	0.913	1.57	0.489	0.537	0.158	0.172	2.79
2A	F	91.8	<0.008	0.207	0.755	1.18	0.374	0.297	0.053	0.101	2.15
2A	F	105.0	<0.072	0.216	0.641	0.958	0.277	0.342	0.087	0.085	1.36
2A	M	47.5	<0.008	0.362	1.05	1.58	0.324	0.253	0.031	0.120	5.68
2A	M	88.0	0.019	0.247	0.733	1.15	0.371	0.308	0.045	0.105	2.51
2A	M	130.2	<0.071	0.218	1.10	1.77	0.575	0.574	0.182	0.088	2.37
2A	M	132.0	<0.071	0.231	1.25	2.32	0.655	0.655	0.188	0.144	4.79
2A	M	140.5	<0.008	0.382	2.26	3.15	0.949	0.702	0.130	0.168	6.23
3A	F	78.2	0.031	0.388	1.08	1.80	0.386	0.250	0.011	0.182	4.33
3A	F	81.1	<0.008	0.203	0.406	0.881	0.211	0.165	0.015	0.077	2.03
3A	F	82.0	0.027	0.361	0.740	1.23	0.300	0.272	0.044	0.127	2.78
3A	F	94.3	0.011	0.336	0.858	1.47	0.349	0.428	0.077	0.303	4.52
3A	F	99.5	<0.071	0.172	1.08	1.43	0.410	0.288	0.072	0.082	3.98
3A	M	94.0	0.040	0.309	0.878	1.15	0.363	0.231	0.033	0.112	3.75
3A	M	102.1	<0.008	0.199	0.498	0.719	0.172	0.162	0.018	0.057	1.57
3A	M	115.7	0.042	0.286	1.11	1.52	0.378	0.370	0.041	0.060	2.41
3A	M	142.0	<0.072	0.293	0.947	1.92	0.599	0.534	0.140	0.106	3.87
3A	M	157.0	0.016	0.385	1.46	2.48	0.631	0.594	0.148	0.105	4.71

Table S3. Sex-based differences by site investigated on a site by site basis for PFOA, PFNA, PFDoA, PFTA, and PFHxS concentrations (log; ng/g) in plasma from American alligators (*Alligator mississippiensis*) sampled at multiple sites in Florida and South Carolina **Bold** indicates significant correlation coefficients. Refer to table 1 and figure 1 for chemical and site abbreviations, respectively.

Site	PFOA	PFNA	PFDoA	PFTA	PFHxS
YK	0.597	0.267	0.917	0.354	**0.009**[b]
KA	0.251	0.228	0.602	0.208	0.465
BI	0.675	0.888	0.175	0.584	0.602
LO	0.175	**0.008**[b]	**0.028**[a]	**0.016**[a]	0.175
WO	0.341	0.866	0.251	0.259	0.347
AP	0.753	0.652	0.917	0.625	0.175
MI	**0.047**[a]	0.365	0.111	0.637	0.903
JR	0.753	0.752	0.347	0.583	0.076
KS	0.117	0.810	0.251	0.222	0.076
TR	0.463	**0.050**[a]	0.117	0.141	**0.009b**
2A	0.142	0.091	0.175	0.97	0.465
3A	0.465	0.853	0.465	0.402	0.175

[a] Correlation is significant at the 0.05 level (2-tailed).

[b] Correlation is significant at the 0.01 level (2-tailed).

Table S4. Site differences in PFDA and PFHxS concentrations (ng/g) in plasma of male and female American alligators (*Alligator mississippiensis*) sampled at multiple sites in Florida and South Carolina. Refer to figure 1 for site abbreviations.

Male

Site	n	PFDA				PFHxS						
YK	5			C	D	A	B	C	D	E		
KA	5				D					E	F	
BI	5	A	B				B	C	D	E	F	
LO	5	A				A	B	C				
WO	5		B	C					D	E	F	
AP	5	A	B					C	D	E	F	
MI	10	A	B									G
JR	5	A	B			A	B	C	D			
KS	5	A	B								F	
TR	5	A	B			A	B					
2A	5	A	B			A	B	C	D			
3A	5	A	B			A						

Female

Site	n	PFDA				PFHxS					
YK	5				D			C	D	E	
KA	5				D					E	
BI	5			C		A	B	C	D		
LO	5	A				A					
WO	5			C			B	C	D	E	
AP	5		B	C		A	B	C	D	E	
MI	5	A	B	C							F
JR	5		B	C		A	B	C	D		
KS	5		B	C						E	
TR	5	A				A	B	C	D		
2A	5	A	B	C		A	B				
3A	5	A	B	C		A	B				

Different letters represent statistically significant differences between groups ($p < 0.05$).

Figure S1. Site PFAA fingerprint for **(A)** male and **(B)** female American alligators (Alligator mississippiensis) sampled in Florida and South Carolina.. Refer to table 1 and figure 1 for chemical and site abbreviations, respectively.

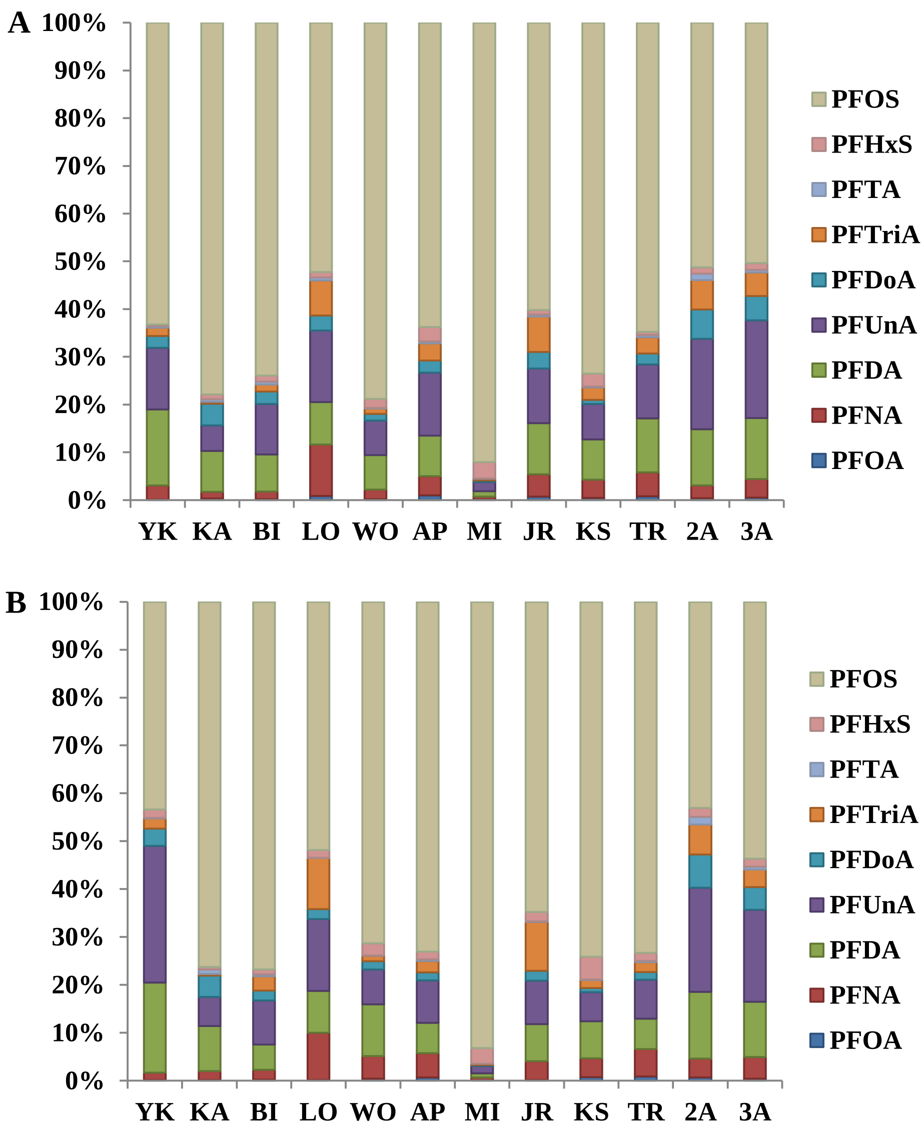


Figure S2. Mean (±SD) concentrations (ng/g) of **(A)** PFDA (p = 0.0003), **(B)** PFUnA (p = 0.021), and **(C)** PFTriA (p = 0.021) in plasma of American alligators (*Alligator mississippiensis*) sampled at multiple sites in Forida and South Carolina.Refer to figure 1 site abbreviations.

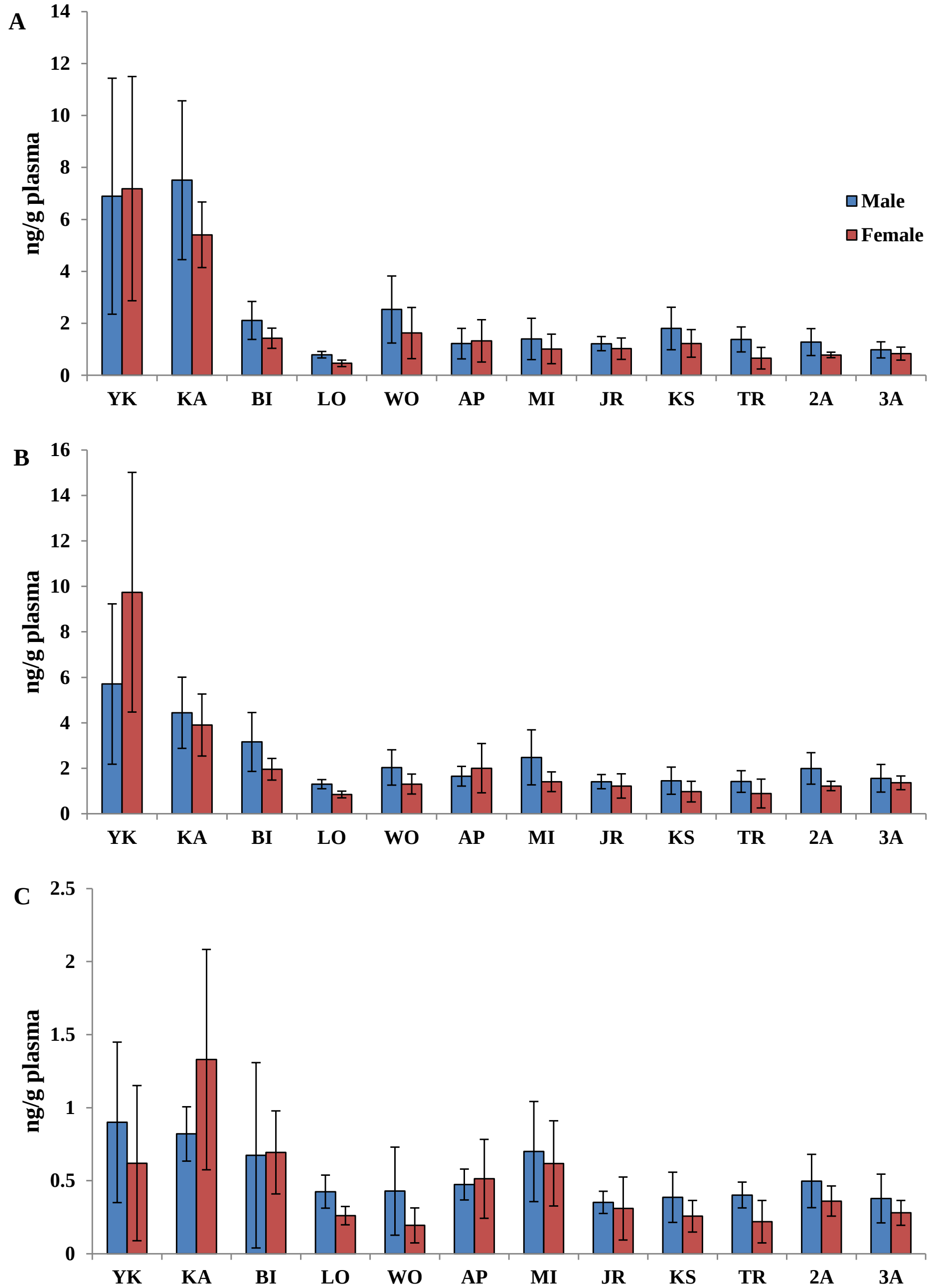


Figure S3. Signifcant ($p < 0.05$) site- and sex-based differences in mean (±SD) concentrations of PFNA, PFTA, PFHxS, PFTA, and PFDoA in plasma of American alligators (*Alligator mississippiensis*) sampled at multiple sites in Florida and South Carolina: **(A)** Lochloosa Lake (LO) had three PFAAs that showed sex-based differences: PFNA ($p = 0.016$), PFTA ($p = 0.032$), and PFDoA ($p = 0.032$), **(B)** the Merritt Island National Wildlife Refuge (MI) site showed sex-based difference in PFOA burden ($p = 0.047$), and **(C)** Lake Trafford (TR) showed sex-based differnces for PFHxS ($p= 0.008$), **(D)** Yawkey (YK) also showed sex-based differences for PFHxS ($p = 0.008$), and **(E)** Lake Trafford (TR) showed sex based-differences in PFNA ($p = 0.050$). Median and interquartile ranges (error bars) represented in **(A)** – **(E)**. Refer to table 1 and figure 1 for chemical and site abbreviations, respectively.

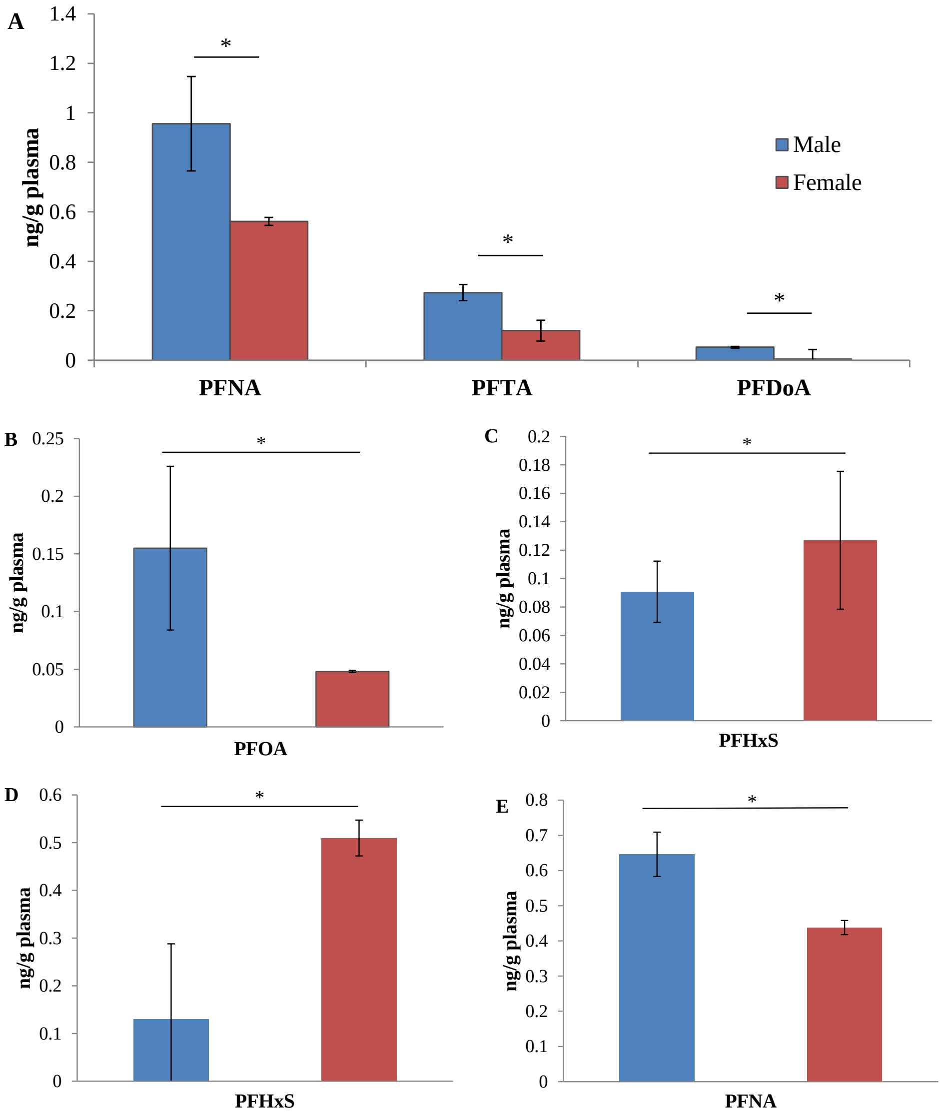
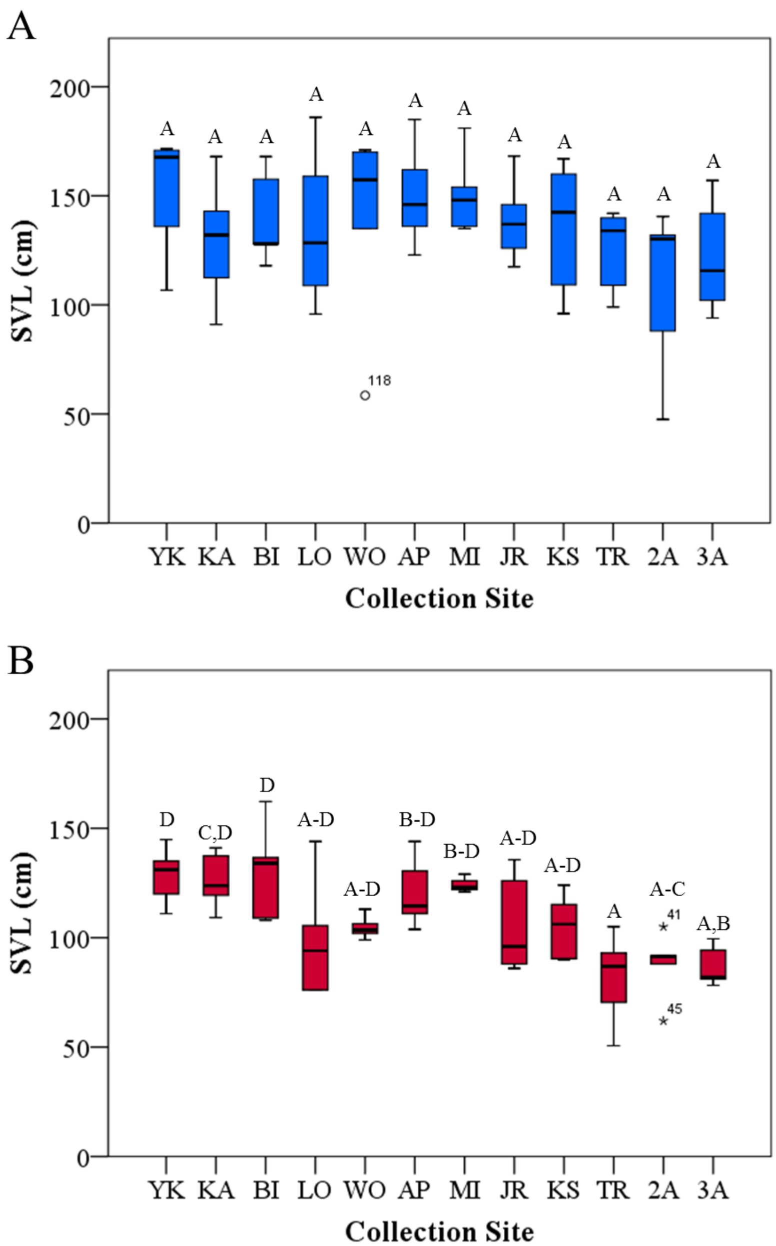


Figure S4. Snout-vent length (SVL) of **(A)** male and **(B)** female American alligators (*Alligator mississippiensis*) sampled at multiple sites in Florida and South Carolina during this study. Refer to figure 1 for site abbreviations.



Different letters represent statistically significant differences between groups ($p < 0.05$).