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Variation in perfluoroalkyl acids in the American alligator (*Alligator mississippiensis*) at Merritt Island National Wildlife Refuge

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Abstract

This study aimed to quantify concentrations of fifteen perfluoroalkyl acids (PFAAs) in the plasma of American alligators (*Alligator mississippiensis*) inhabiting wetlands surrounding the Kennedy Space Center (KSC) in Florida, USA located at Merritt Island National Wildlife Refuge (MINWR). Approximately 10 male and 10 female alligators ($n_{\text{total}} = 229$) were sampled each month during 2008 and 2009 to determine if seasonal or spatial trends existed with PFAA burden. PFOS represented the highest plasma burden (median 185 ng/g) and PFHxS the second highest (median 7.96 ng/g). While no significant seasonal trends were observed, unique spatial trends emerged. Many of the measured PFAAs co-varied strongly together and similar trends were observed for PFOS, PFDA, PFUnA, and PFDoA, as well as for PFOA, PFHxS, PFNA, PFTriA, and PFTA, suggesting more than one source of PFAAs at MINWR. Higher concentrations of PFOS and the PFAAs that co-varied with PFOS were collected from animals around sites that included the Shuttle Landing Facility (SLF) fire house and the Neil Armstrong Operations and Checkout (O&C) retention pond, while higher concentrations of PFOA and the PFAA that co-varied with PFOA were sampled from animals near the gun range and the old fire training facility.

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Sex-based differences and snout-vent length (SVL) correlations with PFAA burden were also investigated.

Keywords

PFAA; PFOS; PFOA; spatial trends; American alligator

1. Introduction

Perfluoroalkyl substances (PFAS) have been manufactured for over 50 years for use in many commercial and industrial-related products such as surfactants, stain repellants, and aqueous film forming foams (AFFF) (Renner, 2006). Nonspecifically, PFAS encompass a vast number of organic and inorganic compounds that contain a carbon-fluoride bond (C-F). These C-F bonds are the unique feature that identify a compound as a PFAS and provide the noted chemical and thermal stability of these compounds (Moody and Field, 2000) that make them heavily desired in industrial and commercial products. Within the PFAS catalog exists a family of highly fluorinated compounds known as perfluoroalkyl acids (PFAAs). Structurally, PFAAs can widely vary, but as a whole PFAAs typically consist of carbon chains of varying length (linear and branched isomers) and an acid functional group. Most importantly, all carbon-hydrogen (C-H) bonds are substituted with C-F bonds (Buck et al., 2011). Two well-studied sub-families of PFAAs are the carboxylic acids and sulfonic acids, and the most commonly known and studied PFAAs are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).

Like other anthropogenic contaminants, PFAAs enter the environment through both point and non-point sources. Non-point sources include storm water runoff from residential areas (Xiao et al., 2012), while point sources include AFFF usage (de Solla et al., 2012) and other industrial and commercially-related sources (Müller et al., 2011; Xiao et al., 2012). While active manufacturing and use of certain PFAAs, notably PFOS and PFOA, have largely ceased due to a voluntary phase-out by industry (Glaser, 2001), several factors contribute to the continued presence of PFAAs in the environment. Firstly, stable C-F bonds prevent chemical, microbial, and photolytic degradation once released into the environment. In addition, stockpiles of previously purchased PFAAs containing products such as AFFFs, continue to be employed and contribute to environmental contamination despite removal from current chemical production lines (Lloyd-Smith and Senjen, 2015). Finally, many precursor chemicals still in production have non-fluorinated structural components that, unlike the C-F bonds, are amenable to microbial or chemical transformation and are attached to a stable perfluorinated chain. These precursors have the potential to degrade into perfluorinated carboxylic and sulfonic acids which can further add to environmental concentrations of PFAAs (Houtz et al., 2013).

When PFAAs released into the environment are consumed by humans and wildlife, they bioaccumulate and biomagnify in food webs (Houde et al., 2011). High trophic level species, such as piscivorous birds, exhibit the highest concentrations of these PFAAs (Giesy and Kannan, 2001). American alligators (*Alligator mississippiensis*) also include fish as a

major dietary component (Rice, 2004) and may therefore accumulate high concentrations of PFAAs as well.

Laboratory studies on mice and rats reveal a wide range of PFAA-related effects including alterations in liver physiology and serum cholesterol, as well as resulting hepatomegaly, wasting syndromes, neurotoxicity, and immunotoxicity (Anderson et al., 2008; Stahl et al., 2011; DeWitt et al., 2012). In addition, PFAAs have also been suggested as possible obesogens, due to their interaction with peroxisome proliferator activated receptors (PPAR) (Grün and Blumberg, 2009). However, species-specific variations in PFAA excretion rates have been observed [2] and the actual mechanism(s) of action of PFAA toxicity is not well understood across species or sex.

Previous studies investigating temporal patterns in PFAA concentrations in both humans and wildlife have shown that these chemicals can persist in plasma for decades and the forms and concentrations detected mirror production rates of specific PFAAs (Glynn et al., 2012; Roos et al., 2014). Shorter seasonal studies reveal additional distinctive trends in PFAA concentrations in surface waters of lakes and rivers (Zhao et al., 2015) as well as polar ice caps [20]. To date, seasonal studies of PFAAs in wildlife tissues is limited. One study on wild male mink found an effect of season on several PFAAs (Persson et al., 2013), while other studies on sea otters (Kannan et al., 2006) and bottlenose dolphins (Houde et al., 2006) found no effect of season on PFAAs. Whether or not tissues of cold blooded animals, such as reptilians, reflect an effect of season on PFAA levels remains to be seen.

Crocodylians (alligators, caimans, crocodiles, gharials) are attractive sentinel species for investigating the accumulation and potential health impacts of PFAAs and other persistent chemicals on wildlife. (Crain and Guillette, 1998; Guillette et al., 2000; Milnes and Guillette, 2008). Due to their high trophic status, long life span, and high site fidelity, crocodylians are susceptible to exposure and accumulation of environmental contaminants released (e.g., chemical application, spill, faulty disposal) or atmospherically deposited in their habitats (Rainwater et al., 2007). While numerous studies have examined accumulation of other persistent environmental contaminants (e.g., organochlorine pesticides, polychlorinated biphenyls, metals) in crocodylians (Campbell, 2003), to date, only four studies have reported PFAAs in these animals (Wang et al., 2013; Bouwman et al., 2014; Christie et al., 2016). Most recently, Bangma et al. (Bangma et al., 2016) found that American alligators (*Alligator mississippiensis*) located within Merritt Island National Wildlife Refuge (MINWR), Florida, have an elevated plasma PFAA burden compared to alligators examined at a number of other sites in Florida and South Carolina (Bangma et al., 2016). It has been proposed that the high concentrations of PFAAs at MINWR may be related to the aeronautic activities of Kennedy Space Center (KSC). In the past, airports and other industrial facilities have shown elevated water concentrations of PFOS downstream of the facilities due to uses of products such as AFFF (de Solla et al., 2012). MINWR was the only site situated on an aeronautic or aviation site of the 12 locations investigated for alligator PFAA burden (Bangma et al., 2016) in that study. Thus, it is not surprising that alligators at MINWR maintained higher PFOS concentrations than the alligators at the other 11 sites investigated in the 2016 study (Bangma et al., 2016).

This study further investigated PFAAs in the plasma from adult American alligators at MINWR in an effort to identify the presence of any spatial and/or seasonal trends in PFAAs and/or concentration in this population. Relationships between PFAA concentrations and alligator sex and body size were also examined as a part of the study objectives.

2. Materials and methods

2.1. Study site

A unique site for wildlife studies, MINWR was established through an agreement between KSC and the U.S. Fish and Wildlife services in 1963. In the agreement, MINWR was established as encompassing all marshland and seashore habitats surrounding the KSC facilities that were not developed for KSC use. Thus, KSC and MINWR are intimately linked together geographically and this environment is comprised of anthropogenic-based landmarks, such as the well-known Vehicle Assembly Building (VAB) and shuttle launch pads, but also local wildlife, such as alligators and birds (Fig. 1).

2.2. Sample collection

From January 2008 to December 2009, adult American alligator plasma samples ($n = 229$) were collected at MINWR adjacent to various aerospace-related activity areas (Fig. 1) as a result of an ongoing collaboration between the Medical University of South Carolina (MUSC) and Integrated Mission Support Service at KSC, FL. Approximately 10 male and 10 female alligators were sampled per month and GPS coordinates were recorded at each alligator capture site for later GIS analysis. Snout-vent length (SVL) was measured for each animal and recorded as a proxy for size and alligators were sexed by cloacal examination of the genitalia (Allsteadt and Lang, 1995). Blood was collected as described by Myburgh et al. (Myburgh et al., 2014) in 8 mL lithium-heparin Vacutainer blood collection tubes (BD, Franklin Lakes, NJ) and centrifuged at 2500 rpm at 4 °C for 10 min. Aliquoted plasma was stored at -20 °C until analysis.

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

2.3. Chemicals

NIST Reference Materials (RMs) 8446 Perfluorinated Carboxylic Acids and Perfluorooctane Sulfonamide in Methanol and RM 8447 Perfluorinated Sulfonic Acids in Methanol were combined to create calibration solutions. Combined, the solution contained a total of 15 PFAAs as follows: perfluorobutyric acid (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), perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), PFOS, and perfluorooctanesulfonamide (PFOSA).

All internal standards (IS) were purchased from Cambridge Isotope Laboratories (Andover, MA), RTI International (Research Triangle Park, NC), and Wellington Laboratories (Guelph, Ontario), in order to create an IS mixture that comprised of a total of eleven isotopically labeled PFAAs. The mixture is as follows: [$^{13}\text{C}_4$]PFBA, [$^{13}\text{C}_2$]PFHxA, [$^{13}\text{C}_8$]PFOA, [$^{13}\text{C}_9$]PFNA, [$^{13}\text{C}_9$]PFDA, [$^{13}\text{C}_2$]PFUnA, [$^{13}\text{C}_2$]PFDaA, [$^{18}\text{O}_2$]PFBS, [$^{18}\text{O}_2$]PFHxS, [$^{13}\text{C}_4$]PFOS, and [$^{18}\text{O}_2$]PFOSA.

2.4. Sample preparation

Plasma samples were extracted using a method previously described in detail in 2011 by Reiner et al. (Reiner et al., 2011). Briefly, approximately 1 mL of each alligator plasma sample and three SRM 1958 aliquots were spiked with approximately 600 μL IS mixture. The exact amount of IS mixture was gravimetrically recorded for each sample prior to extraction with 4 mL acetonitrile. Samples were further purified in methanol using an Envi-Carb cartridge (Supelco, Bellefonte, PA) and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Samples were analyzed using an Agilent 1100 High Performance Liquid Chromatography 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. An Agilent Zorbax Eclipse Plus C18 analytical column (2.1 mm $\times$ 150 mm $\times$ 5 μm) was used for PFAA separation. Each sample run involved a ramping LC solvent gradient with methanol and de-ionized water both containing 20 mmol/L ammonium acetate (Reiner et al., 2011). Two multiple reaction monitoring (MRM) transitions for each PFAA were monitored to ensure no interferences. One MRM was employed for quantitation and the other transition was used for confirmation (Reiner et al., 2011).

2.5. Quality control

All samples were processed alongside quality control material NIST SRM 1958 and blanks to determine the efficacy of the method. The PFAA concentrations of SRM 1958 processed during our extraction met established values reported on the Certificate of Analysis (CoA). Measured compounds were considered 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.

2.6. Statistical methods

All statistical analyses were performed using IBM SPSS statistic 22 (Armonk, NY: IBM Corp.). Statistical tests were performed for PFOA, PFNA, PFDA, PFUnA, PFDaA, PFTriA, PFTA, PFHxS, and PFOS which were detected in 89 % to 100 % of samples (Table 1). The remaining PFAAs (PFBA, PFPeA, PFHxA, PFHpA, PFBS, and PFOSA) were detected in less than 15 % of the samples and were excluded from statistical analyses. For those PFAAs included in statistical analyses, compounds less than the RL were set equal to half the RL prior to running the statistical tests (Keller et al., 2005). Reporting limits ranged from 0.007 ng/g to 0.056 ng/g.

Friedman's test was used as a randomized complete block design to determine monthly variations in plasma PFAA concentrations with non-normally distributed concentration values (Bhujel, 2009). Month 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 non-parametric tests employed for data analysis of sex-based differences included Mann-Whitney U tests when the data remained non-normal after log transformation. Spearman correlations were used when applicable for correlative measures. ArcGIS 10.3 (Esri, Redlands, CA) was employed to construct spatial PFAA trends in alligator plasma at MINWR.

3. Results and discussion

Nine of the fifteen PFAAs investigated were detected regularly in the alligator plasma samples ($n = 229$) and they are as follows (in order of abundance): PFOS, PFHxS, PFUnA, PFDA, PFNA, PFTriA, PFDaA, PFOA, and PFTA (Table 1). These nine PFAAs resulted in unique fingerprints for MINWR (Supplemental Information, Fig. S1). As expected, PFOS, which is commonly found in environmental samples, was the highest measured PFAA at MINWR with a median of 185 ng/g (Houde et al., 2011). However, the PFOS measured in alligators from MINWR had a large range, 6.06 ng/g – 2140 ng/g, revealing the highest PFOS concentration yet measured in a crocodilian species (Wang et al., 2013; Bouwman et al., 2014; Christie et al., 2016). PFHxS was the second highest PFAA measured with a median of 7.96 ng/g and a range of < 0.008 ng/g to 161 ng/g. These two PFAAs (PFOS and PFHxS) have been shown to have a uniquely high signature in MINWR alligator plasma compared to alligators at several other sites in Florida and South Carolina (Bangma et al., 2016), suggesting local sources in close proximity to MINWR containing a high proportion of these two PFAAs. Interestingly, this is not the first study to show low or non-detectable concentrations of PFOA compared to other PFAAs in crocodilian plasma (Wang et al., 2013; Bangma et al., 2016). Wang et al. (Wang et al., 2013) found a mean PFOA concentration of 0.02 ng/ml in captive Chinese alligators (*Alligator sinensis*), while Bangma et al. (Bangma et al., 2016) reported a mean PFOA concentration of 0.116 ng/g in American alligators from multiple sites in Florida and South Carolina. It is possible crocodilians have an ability to excrete PFOA more effectively than humans or other wildlife species, preventing bioaccumulation in crocodilian plasma. An alternate possibility is PFOA in crocodilians may segregate more effectively into other blood rich organs such as the liver and kidney. A third possibility is the lack of PFOA in the fish they consume as a part of their diet and thus a lack of bioaccumulation through the food web. Several studies have previously found that PFOA is often low or below the limits of detection in fish species in both the Great Lakes and the Mississippi River (Ye et al., 2008; Delinsky et al., 2010).

3.1. Sex differences

No sex-based differences in PFAA concentrations in alligator plasma were observed by month (data not shown). However, when all alligators were pooled by sex and regardless of month, males ($n = 117$) exhibited significantly higher concentrations of PFTA, PFTriA, and PFHxS ($p < 0.001$) compared to females ($n = 112$). Males also exhibited higher concentrations of PFOA and PFNA ($0.05 < p < 0.10$) (Table 2). However, the adult male

alligators sampled at MINWR in this study had a significantly greater SVL than females ($p < 0.001$) (Supplemental Information, Fig. S2). To account for this sex-specific difference in body size, SVL data from 1,077 individual alligator captures (recapture animals not included) at MINWR from September 2006 to the end of November 2015 were used to length-adjust data. The resulting length-adjusted data showed few differences from the non-adjusted data. The one exception was a trending higher ($0.05 < p < 0.10$) PFOA concentration in males than females using the non-adjusted data to no significant difference between the sexes using the adjusted data (Table 2).

The sex-based differences observed at MINWR reveal different results than have been observed in previous crocodilian studies (Supplemental Information, Table S1). We hypothesize that our large sample size at MINWR ($n = 229$) has provided increased sensitivity for detecting sex-based differences in PFAAs not previously observed in adult crocodilians, such as PFTriA and PFHxS. However, for other PFAAs like PFOS, for which sex-based differences have been observed in other crocodilian studies, no sex-based differences were observed for this study. We hypothesize that high concentrations of point source contamination of PFOS at MINWR is potentially masking sex-based differences due to a larger active input of PFOS from point sources that mask the slower excretion or bioaccumulative differences between the sexes. One common finding among the few PFAA-crocodilian studies to date (including the present study) is when significant sex-based differences in PFAA concentrations are found, males consistently exhibit higher plasma PFAA concentration than females.

Sex-based differences (i.e., males with higher concentrations than females) observed for certain PFAAs in crocodilians could be due to several factors. First, crocodilians may exhibit a differential clearance in PFAAs between males and females, as has been observed in rats (Kudo et al., 2001), mice (Gannon et al., 2011), and other mammals (Han et al., 2012). Second, female crocodilians may off-load PFAAs during oviposition, thereby reducing their PFAA body burden compared to males in the same environment. This hypothesis is supported by studies which have shown measurable concentrations of specific PFAAs in herring gull (*Larus argentatus*) eggs in North America (Gebbink and Letcher, 2010) and Nile crocodile (*Crocodylus niloticus*) eggs in South Africa (Bouwman et al., 2014), 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 (Joanen and McNease, 1970; Joanen and McNease, 1972; Hutton, 1989; Tucker et al., 1997). In such cases, differences of prey availability and contamination between and among habitats within a site could result in different PFAA exposures in males and females.

3.2. PFAA Correlations

Since sex based differences were observed for a number of the PFAAs measured, PFAA correlations were looked at separately for each sex. Significant correlations were observed between certain PFAAs measured in the plasma, suggesting possible similar sources of PFAA contamination at MINWR (Table 3). Some correlative relationships between the co-varying PFAAs were stronger than others, which we infer to suggest their likelihood to be

from similar local point sources. PFOS, which is found in high concentrations in alligators from MINWR, likely as a result of point source of contamination in the area, is highly correlated with PFDA, PFUnA, and PFDoA (Table 3). These strong correlations suggest but do not guarantee that similar point sources that are responsible for PFOS levels are also likely contributing to concentrations of these other highly correlated PFAAs at MINWR. This could explain why sex-based differences were not found for PFOS, PFDA, PFUnA, and PFDoA at MINWR in contrast to other studies (Table S1) due to a potential point source contamination of these chemicals masking a more subtle sex-based difference. PFHxS also co-varies well with PFOS but not as strongly with PFDA, PFUnA, and PFDoA. In general, PFHxS co-varies more highly with PFNA, PFOA, and PFTA and co-varies less with PFDA, PFUnA, and PFDoA, (Table 3) this particular result suggests multiple possible sources for the various PFAAs.

Correlations comparing both male and female SVL to PFAAs resulted in a number of significant positive correlations for PFOA, PFNA, PFTriA, PFTA (females only), and PFHxS (Table 4). Overall, males exhibited stronger correlations between PFAA burden and SVL when compared to females. The strongest correlations for males were with PFTA ($\rho = 0.546$, $p < 0.01$) followed closely by PFTriA ($\rho = 0.462$, $p < 0.01$). In contrast, the highest correlation coefficients for female SVL and PFAA burden was PFOA ($\rho = 0.412$, $p < 0.01$), closely by PFNA ($\rho = 0.276$, $p < 0.01$). No correlations were seen between SVL and the same PFAAs shown to co-vary with PFOS, PFDA, PFUnA, and PFDoA. We hypothesize no correlations were seen between these PFAAs and SVL due to a masking by active point source contamination at MINWR before or during 2008 and 2009.

3.3. Seasonal trends

No statistically significant trends were observed for the investigated PFAAs in alligator plasma across seasons or months in either females or males at MINWR. It is possible the high point source contamination at MINWR is masking a possibly subtle seasonality trend similarly to masking a sex-based difference as well as PFAA and SVL correlations as described earlier. In attempts to assess this, extreme values for PFOS (> 500 ng/g) were removed from the data set to evaluate trends with a reduced effect from the point source contamination on the data. Since the majority of animals were sampled from 2009, the data were also restricted to samples collected in 2009 to assess seasonality for that year (resulting $n = 152$). In doing so, an interesting but non-significant trend was seen in the averages of monthly PFOS that were similar to monthly weather averages in Titusville, FL for 2009 (Supplemental Information, Fig. S3). As the year progressed and the weather warmed, PFOS values increased slightly (non-significantly) until peak temperatures in August, then gradually decreased as temperatures dropped in the fall and winter months. One hypothesis for this potential trend is that in warmer months there is increased bacterial breakdown of precursor PFAAs resulting in an increase in PFOS concentrations (Liu and Mejia Avendaño, 2013). While not completely removed from the influences of point source contamination, this trend warrants further investigation into PFOS seasonality influences and a site without point source contamination might be better suited to unraveling this question.

3.4. Spatial Trends

Because American alligators exhibit high site fidelity (Fujisaki et al., 2014), spatial trends were investigated for plasma PFOS, PFHxS, PFUnA, PFDA, PFNA, PFTrIA, PFDoA, PFOA, and PFTA using ArcGIS. We were able to assess MINWR areas for the existence of possible point sources of contamination using samples collected during 2008 and 2009 at MINWR. Similar trends were observed for the PFAAs that co-varied strongly; PFOS, PFDA, PFUnA, and PFDoA, as well as for PFOA, PFHxS, PFNA, PFTrIA, and PFTA.

Even with a large range of plasma PFOS concentrations (6.06 ng/g – 2,140 ng/g), PFOS concentrations in the 229 American alligators sampled during 2008 and 2009 at MINWR exhibited a distinct spatial pattern with several focal hot spots across MINWR (Fig. 2A), which suggests potential sources of PFOS within MINWR during or before 2008 and 2009. In addition, continued examination of Fig. 2A suggested the existence of more than one potential source location of PFOS. For example, alligators with some of the highest plasma PFOS were captured adjacent to the Shuttle Landing Facility (SLF) fire house, as well as the Neil Armstrong Operations and Checkout (O&C) retention pond. Past use of AFFF at the SLF fire house is a possible PFOS contributor for exposure to alligators inhabiting the immediate area.

Additionally, a number of alligators exhibiting high plasma PFOS concentrations were captured around a cluster of facilities located at MINWR that include the old water treatment plant, hazardous storage area, Vehicle Assembly Building (VAB), solid rocket processing, and the high pressure gas facility. Within this cluster of buildings, the concentration of high PFOS appears to most closely associate with the hazardous storage area as well as the VAB; while alligators have high site fidelity, alligators do move short distances and may travel around these buildings, making GIS less definitive for such a tight cluster of landmarks. In general, the largest overall area with elevated PFOS concentrations seems to have been situated in and around the Banana River possibly due to PFOS runoff from point sources such as past use of AFFF.

The spatial trend of plasma PFOA (Fig. 2B) with a smaller range (<0.008 ng/g to 7.27 ng/g) exhibited a vastly different pattern at MINWR than PFOS. With uniformly low concentrations across most of MINWR, only a few alligators show PFOA concentrations in the highest quintile (ranging from 3.20 ng/g - 4.00 ng/g) and even those few alligators with higher PFOA concentrations reside in differing locations at MINWR than alligators with higher PFOS. For example, the two sites adjacent to the capture location for alligators possessing the highest concentrations of PFOA are the gun range and the old fire training center. Ammunition manufacturers routinely employ metallic plating processes that include the use of PFOA, which leaves trace amounts of PFOA in the ammunition (Feldstein, 2012). We hypothesize contributions of PFOA around the gun range may be a result of PFOA leaching from spent ammunition.

4. Conclusions

This study examined PFAA concentrations in plasma from American alligators at MINWR in 2008 and 2009, a timeframe when PFAAs were present in many commercially available

products. Nine of the fifteen PFAAs investigated were detected regularly in plasma: PFOS, PFHxS, PFUnA, PFDA, PFNA, PFTrIA, PFDoA, PFOA, and PFTA. PFOS represented the highest plasma burden (median 185 ng/g) and PFHxS the second highest (median 7.96 ng/g). Of those regularly detected PFOS, PFDA, PFUnA, and PFDoA, co-varied strongly with one another, while PFOA, PFHxS, PFNA, PFTrIA, and PFTA co-varied strongly with one another. Sex-based differences as well as stronger SVL and PFAA correlations were more commonly observed for PFAAs that co-varied with PFOA, while PFAAs that co-varied with PFOS showed no sex-based differences and weaker SVL and PFAA correlations. We hypothesize this is due to multiple sources of contamination at MINWR during or before 2008 and 2009 with the potentially most active (or previously active) source being the source containing PFOS, PFDA, PFUnA, and PFDoA. Strong spatial patterns were seen for both groups of PFAAs furthering our understanding of PFAA distribution in the alligators across MINWR and possibly providing clues regarding the sources of PFAA contamination on the site. It is important to note that at the time of this study potential sources, like AFFFs, contained PFASs and were legal to use. A follow-up study gathering more contemporary samples from MINWR potentially would show different patterns of PFAAs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Merritt Island National Wildlife Refuge (MINWR) geographical location and Kennedy Space Center (KSC) facilities around MINWR. **(A)** Launch pad 39 B, **(B)** Launch pad 39 A, **(C)** Shuttle landing facility (SLF) fire house, **(D)** Crawler processing area, **(E)** High pressure gas facility, **(F)** Hazardous storage area, **(G)** Solid rocket processing area, **(H)** Old water treatment facility, **(I)** Vehicle assembly building, **(J)** Gun range, **(K)** Dump, **(L)** Old fire training center, and **(M)** Operations and Checkout retention pond.

Fig. 2. Spatial distribution of (A) PFOS and (B) PFOA in American alligator plasma (n = 229) at MINWR during 2008–2009.

Table 1

Perfluoroalkyl acid (PFAA) concentrations (ng/g wet mass) in plasma from American alligators at Merrit Island National Wildlife Refuge (MINWR). Values were calculated with half the RL substituted for non-detects as described in the methods section, but values shown as “<” a specified number describe the actual RL.

	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFHxS	PFOS
n	229	229	229	229	229	229	229	229	229
% > RL	89	99	99	100	91	100	71	96	100
Median (ng/g)	0.14015	0.941	1.455	2	0.379	0.6273	0.106	7.955	185
Range (ng/g)	<0.008 – 7.27	<0.009 – 9.51	<0.008 – 16.2	0.464 –9.52	<0.008 – 11.3	0.075 – 4.02	<0.007 – 3.88	<0.008 – 161	6.06 – 2143

%> RL indicates the number of samples above the reporting limit (RL)

Table 2

Sex-based probability values (p values) in PFAA concentrations in plasma of American alligators at Merritt Island National Wildlife Refuge (MINWR) (n_{females} = 112, n_{males} = 117).

Sex based differences	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFHxS	PFOS
MINWR	0.070	0.078	NS	NS	NS	p < 0.001	p < 0.001	p < 0.001	NS
MINWR length adjusted	NS	0.064	NS	NS	NS	p < 0.001	p < 0.001	p < 0.001	NS

Trending males higher (0.05 < p < 0.10)

Males significantly higher than females (p < 0.05)

NS = Not Significant

Table 3

Pearson correlation coefficients between various plasma PFAAs for alligators at MINWR ($n_{\text{male}} = 117$, $n_{\text{female}} = 112$). **Bold** indicates significance ($p < 0.05$).

Female (n = 112)	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFHxS	PFOS
PFOA	-	0.622**	0.454**	0.346**	0.431**	0.524**	0.341**	0.571**	0.380**
PFNA		-	0.615**	0.456**	0.396**	0.475**	0.262**	0.582**	0.576**
PFDA			-	0.892**	0.669**	0.582**	0.283**	0.453**	0.731**
PFUnA				-	0.701**	0.554**	0.243**	0.377**	0.683**
PFDoA					-	0.762**	0.514**	0.264**	0.392**
PFTriA						-	0.676**	0.273**	0.323**
PFTA							-	0.140	0.095
PFHxS								-	0.749**
PFOS									-

Male (n = 117)	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFHxS	PFOS
PFOA	-	0.671**	0.185*	-0.015	0.129	0.459**	0.464**	0.752**	0.320**
PFNA		-	0.573**	0.356**	0.339**	0.505**	0.424**	0.579**	0.553**
PFDA			-	0.855**	0.613**	0.304**	0.119	0.146	0.722**
PFUnA				-	0.657**	0.279**	-0.005	-0.021	0.565**
PFDoA					-	0.528**	0.253**	0.166	0.463**
PFTriA						-	0.738**	0.461**	0.330**
PFTA							-	0.512**	0.143
PFHxS								-	0.386**
PFOS									-

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 4

Pearson correlation coefficients between snout-vent-length (SVL) and PFAS concentrations in plasma for male (n = 117) and female (n = 112) alligators at Merritt Island National Wildlife Refuge (MINWR). **Bold** indicates significance ($p < 0.05$).

	MINWR	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFHxS	PFOS
Female	0.412 ^{**}	0.276 ^{**}	0.010	-0.169	-0.069	0.219 [*]	0.143	0.240 [*]	-0.002	
Male	0.414 ^{**}	0.183 [*]	-0.201 [*]	-0.248 ^{**}	0.004	0.462 ^{**}	0.546 ^{**}	0.421 ^{**}	-0.020	

^{**} Correlation is significant at the 0.01 level (2-tailed).

^{*} Correlation is significant at the 0.05 level (2-tailed).