Published in final edited form as:

J Environ Sci (China). 2017 November ; 61: 24–30. doi:10.1016/j.jes.2017.05.045.

Analysis of PFAAs in American alligators part 1: Concentrations in alligators harvested for consumption during South Carolina public hunts

Jessica J. Tipton¹, Louis J. Guillette Jr², Susan Lovelace¹, Benjamin B. Parrott³, Thomas R. Rainwater⁴, and Jessica L. Reiner^{5,*}

¹College of Charleston, Charleston, SC 29424, USA

²Medical University of South Carolina, Department of Obstetrics and Gynecology, Charleston, SC 29425, USA

³University of Georgia, Odum School of Ecology, Savannah River Ecology Laboratory, Jackson, SC 29831, USA

⁴Tom Yawkey Wildlife Center & Belle W. Baruch Institute of Coastal Ecology and Forest Science, Clemson University, P.O. Box 596, Georgetown, SC 29442, USA

⁵National Institute of Standards and Technology, Chemical Sciences Division, Hollings Marine Laboratory, Charleston, SC 29412, USA

Abstract

Environmental contamination resulting from the production or release of harmful chemicals can lead to negative consequences for wildlife and human health. Perfluorinated alkyl acids (PFAAs) were historically produced as protective coatings for many household items and currently persist in the environment, wildlife, and humans. PFAAs have been linked to immune suppression, endocrine disruption, and developmental toxicity in wildlife and laboratory studies. This study examines the American alligator, *Alligator mississippiensis*, as an important indicator of ecosystem contamination and a potential pathway for PFAA exposure in humans. Alligator meat harvested in the 2015 South Carolina (SC) public hunt season and prepared for human consumption was collected and analyzed for PFAAs to determine meat concentrations and relationships with animal body size (total length), sex, and location of harvest. Of the 15 PFAAs analyzed, perfluorooctane sulfonate (PFOS) was found in all alligator meat samples and at the highest concentrations (median 6.73 ng/g). No relationship was found between PFAA concentrations and total length or sex. Concentrations of one or all compounds varied significantly across sampling locations, with alligators harvested in the Middle Coastal hunt unit having the highest PFOS concentrations (median 16.0 ng/g; $p = 0.0001$). Alligators harvested specifically from Berkley County, SC (located in the Middle Coastal hunt unit) had the highest PFOS

* Corresponding author: jessica.reiner@nist.gov (Jessica L. Reiner).

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jes.2017.05.045>.

Publisher's Disclaimer: Disclaimer

Publisher's 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.

concentrations and the greatest number of PFAAs detected ($p < 0.0001$). The site-specific nature of PFAA concentrations in alligator meat observed in this study suggests a source of PFAA contamination in Berkley County, SC.

Keywords

Perfluorinated alkyl acids; American alligators; South Carolina; Perfluorooctane sulfonate (PFOS); Dietary exposure

Introduction

Perfluorinated alkyl acids (PFAAs) are synthetic chemicals characterized as having C–F bonds of varying chain length and have been historically used in the production of many common household items (Prevedouros et al., 2006). The water and stain resistant properties of PFAAs have led to their use as surfactants in many commercial products. Additionally, PFAAs and their precursors can be found in aqueous film forming foams, carpets, non-stick cookware, and paper used for food packaging (Prevedouros et al., 2006). PFAAs are stable because of the strong carbon–fluorine bond and persistent in the environment. Chain lengths equal to or greater than 8 carbons are known to accumulate in ecosystems and wildlife (Butt et al., 2008). PFAAs have been found in rainwater, dust, fresh and saltwater bodies, and are believed to be capable of long-range transport by ocean currents and atmospheric transport (Armitage et al., 2006, 2009; Yamashita et al., 2008). Concern about environmental accumulation and potential human health effects has led to strict regulations on the production of PFAAs in the United States and a strategic plan for a phase-out of production was planned for completion in 2015 (EPA, 2000). PFAAs have an extremely long half-life in humans and the environment and are resistant to breakdown from many thermal and chemical processes (Olsen et al., 2012).

PFAAs have been measured in wildlife and humans around the world. The two most common PFAAs found in the environment and wildlife are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). The stability and long-range transport potential of PFAAs and their precursors has contributed to elevated levels of these contaminants in Arctic wildlife, far removed from point sources (Butt et al., 2010). Biomagnification of PFAAs in wildlife has been well recorded, with PFOS concentrations positively related to trophic level and risk of negative health effects (Keller et al., 2012; Muller et al., 2011; Loi et al., 2011; Yordy et al., 2013). Marine mammal studies have linked PFAA exposure to altered renal and hepatic function, as well as immune suppression (Fair et al., 2013). Studies conducted on laboratory animals have also shown immune suppression and increased mortality in PFOS exposed mice (Guruge et al., 2009), decreases in reproductive success in zebrafish from estrogenic effects after exposure to perfluorononanoic acid (PFNA) (Jantzen et al., 2016; Zhang et al., 2016), and developmental abnormalities and liver damage in clawed frogs exposed to PFAAs (Kim et al., 2013).

The impacts on human health from exposure to PFAAs need further investigation; however, current research suggests that exposure to PFAAs could result in endocrine disruption (Kjeldsen and Bonefeld-Jørgensen, 2013), developmental toxicity (Kjeldsen and Bonefeld-

Jørgensen, 2013), and immune suppression (Grandjean et al., 2012). Most human exposure to PFAAs in the United States (U.S.) is through ingestion of contaminated food or drinking water (Lindstrom et al., 2011). Exposure of high risk populations, such as pregnant women and children, is especially concerning due to the nature of health implications from exposure to PFAAs, including impacts to development and immune function.

Charleston Harbor in South Carolina (SC), U.S., is a potential hotspot for PFAA contamination. A study examining PFAA levels in bottlenose dolphins (*Tursiops truncatus*) found some of the highest serum concentrations reported in marine mammals, at similar levels to humans occupationally exposed (Fair et al., 2012). Estuarine sediments tested from the Charleston Harbor watershed were found to have the highest PFAA levels of any urban area recorded in the U.S., with levels increasing from 2004 to 2014 (White et al., 2015). Despite the alarming levels of PFOS and PFOA concentrations being reported in the sediment and wildlife in waterbodies of SC, few studies have examined PFAA contaminant exposure of other aquatic and terrestrial species (Bangma et al., 2017a; Yordy et al., 2013). PFAA accumulation in apex predators such as bottlenose dolphins and contaminant input from unknown sources provide an impetus for a closer investigation of SC wildlife potentially at risk.

Few studies have been conducted on PFAA contamination levels in reptiles (Keller et al., 2012; Wang et al., 2013; Christie et al., 2016; Bangma et al., 2017a, 2017b), and all of these have used blood as a focal tissue for analysis; none have examined PFAA concentrations in other tissues, including those potentially consumed by other wildlife or humans. The American alligator (*Alligator mississippiensis*) is a highly sought after wild game species for which a hunting season has recently (2007) been established in SC. Alligators are apex predators in many freshwater and brackish water ecosystems and play a key role in maintaining a balanced aquatic food web (Nifong and Silliman, 2013). The American alligator can be used as a sentinel species and indicator of contaminants within an ecosystem due to its long lifespan, non-migratory range, and high trophic status (Milnes and Guillette, 2008). There is no current research available on PFAA concentrations in alligator meat harvested in SC's recreational hunts and collected for consumption despite high meat yields from hunts in 2013 (> 11,000 lb) and 2015 (> 9000 lb) (Butfiloski, 2014, 2015). Obtaining exposure information for harvested alligators is critical in determining areas of environmental concern, negative impacts on wildlife health, and potential exposure to humans through consumption of harvested meat. In this study we sampled 43 American alligators recreationally harvested throughout the SC coastal plain during the 2015 public hunt season to determine the concentration of PFAAs in tail meat collected for consumption. Additionally, we examined the relationships between PFAA concentration in tail meat and alligator body size, sex, and location of harvest.

1. Materials and methods

1.1. Sample collection

Tail meat samples (approximately 500 g) were collected opportunistically during the SC recreational hunt season from September 12, 2015 to October 10, 2015 at a local wild game meat processor. Each sample was consistently collected by a licensed processor from the

base (anterior end) of the tail using a clean filet knife. A total of 43 samples were collected, individually wrapped in methanol rinsed tin foil, placed on ice in the field, and later stored at -80°C until analysis. Tail muscle tissue was selected based on its high rate of consumption compared to meat from other parts of the body and subsequent relevance to potential contaminant transfer to human consumers. Morphometric data were obtained prior to alligator processing and sample collection, and any health abnormalities were noted. Sex was determined by cloacal examination of the genitalia (Allsteadt and Lang, 1995). Body measurements included total length (TL; distance from the anterior tip of the snout to the posterior tip of the tail, measured along the dorsal surface), snout-vent length (distance from the tip of the snout to the posterior margin of the third caudal whorl of scales, measured dorsally), and tail girth (measured at the base of the tail at the posterior margin of the third caudal whorl of scales) (Platt et al., 2009, 2011; Wilkinson et al., 2016). Tail meat samples were labeled with the associated tag number provided to each hunter by the state, and the collection date. Harvest locations (Fig. 1) were obtained from in-person and telephone surveys with the permit tag holder and from the South Carolina Department of Natural Resources (SC DNR) hunt report database. Harvest locations obtained from the SC DNR database were checked with self-reported locations to validate reliability. Surveys also collected information on other game species hunted in SC, as well as meat and game fish consumption by hunters and their families. The survey procedure was approved by the College of Charleston Institutional Review Board for Human Research Participant Protections and hunter identity was kept confidential.

1.2. 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: perfluorobutyric acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, 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).

Internal standards (ISs) were purchased from Cambridge Isotope Laboratories (Andover, MA), RTI International (Research Triangle Park, NC), and Wellington Laboratories (Guelph, Ontario) to create an IS mixture comprised of eleven isotopically labeled PFAAs, and they were 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, $^{13}\text{C}_2$ -PFDoA, $^{18}\text{O}_2$ -PFBS, $^{18}\text{O}_2$ -PFHxS, $^{13}\text{C}_4$ -PFOS, and $^{18}\text{O}_2$ -PFOSA.

1.3. Chemical analysis

Tail meat samples were analyzed for PFAA concentrations using methods previously described (Reiner et al., 2012). Briefly, samples were removed from storage and allowed to thaw before all edges were trimmed using a sterilized, methanol rinsed scalpel. Samples were extracted using 0.01 mol/L KOH in methanol twice and furthered cleaned using graphitized carbon. Quantification of 15 PFAAs was completed using liquid chromatography

tandem mass spectrometry (LC–MS/MS; Agilent 1100 HPLC interfaced to API 4000, Applied Biosystems-MDS Sciex). Quality assurance and control methods included blanks and Standard Reference Material (SRM) 1947 Lake Michigan Fish Tissue that were extracted alongside tail meat samples. The concentrations of PFOS, PFNA, PFDA, PFUnA, and PFTrIA in SRM 1947 agreed with values reported on the Certificate of Analysis. Reporting limits (RLs) were determined as the maximum value of either the average mass (in ng) measured in the extract plus 3 times the standard deviation of the blanks or the lowest calibrant detected, all divided by the mass (in grams) of extracted sample.

1.4. Statistical analysis

Statistical analysis was performed using Minitab 17 and SMS software for compounds detected in greater than 70% of the samples. Descriptive statistics were performed to determine the mean, median, and range for PFNA, PFDA, PFUnA, PFDoA, PFTrIA, PFTA, PFHxS, PFOS, and PFOSA concentrations in the 43 tail meat samples. A regression analysis was used to examine the relationship between TL and PFAA concentration, accounting for sex, and a Student's *t*-test was used to determine relationships between sex and PFAA concentration. Log transformation was used for PFDA, PFTrIA, and PFOS to improve distribution and model assumptions, and parametric tests were used for analysis. Non-parametric tests were used for PFUnA, PFDoA, and PFHxS, as these compounds were not normally or log normally distributed. Differences in PFAA concentrations based on location of harvest were examined using a one-way analysis of variance (ANOVA). Harvest locations were analyzed two ways. The first being based on their geographically designated hunt unit (Fig. 1) and also by the county in which the alligator was harvested. When PFAA concentrations fell below the RL, they were replaced with half the RL.

2. Results and discussion

PFDA, PFUnA, PFDoA, PFHxS, and PFOS concentrations were above the RL in all of the study samples (Table 1). PFOS was found in the highest concentration for all samples (median 6.73 ng/g; range 1.02–36.4 ng/g). Previous studies on PFAA levels in wild reptiles have reported similar results with PFOS found frequently and at the highest concentrations (Bangma et al., 2017a; Keller et al., 2012). Although few studies have examined PFAAs in reptiles, those on crocodilians report higher PFOS concentrations in blood serum samples than were found in muscle tissue in this study, with an average PFOS concentration of 23.3 ng/g in wild Nile Crocodiles (*Crocodylus niloticus*) (Christie et al., 2016) and 28.7 ng/g in captive Chinese Alligators (*A. sinensis*) (Wang et al., 2013). Keller et al. (2012) found similar PFOS concentrations in the plasma of Kemps Ridley (*Lepidochelys kempii*) and Hawksbill (*Eretmochelys imbricata*) sea turtles sampled from the Atlantic Coast of the U.S. with median concentrations of 10.8 and 11.9 ng/g, respectively. The maximum PFOS concentration found was 35.0 ng/g in a Kemps Ridley sea turtle, a species that occupies a high trophic level; this is a similar maximum PFOS concentration to that found in the present study. A recent study describing PFAA concentrations in the plasma of American alligators sampled from SC and Florida found slightly higher PFOS concentrations than reported in this study (median 11.2 ng/g; range 1.36–452 ng/g) (Bangma et al., 2017a). However, these prior studies examined PFAA concentrations in blood (serum and plasma)

while the present study examined muscle, which may contain lower PFAA concentrations than the former due to differential partitioning of PFAAs in the body (Ahrens et al., 2009).

Conflicting results have been observed in wildlife studies examining the relationship between PFAA concentrations and age as well as sex (Bangma et al., 2017a; Christie et al., 2016; Fair et al., 2012; Wang et al., 2013). In this study, no significant relationship was observed between any PFAAs and alligator TL or sex ($p > 0.05$). In a previous study examining PFAAs in captive Chinese alligators, the highest PFOS concentrations were found in the “youngest” animals, and males exhibited higher concentrations than females (Wang et al., 2013). PFAA concentrations in a recent study examining American alligator plasma samples described a similar relationship with males exhibiting higher concentrations of some PFAAs and PFAA burden increasing with body size (Bangma et al., 2017a). Although not directly studied in reptiles, laboratory studies of mammals have shown sex-based differences in clearance of PFAAs (Gannon et al., 2011; Kudo et al., 2001). Previous studies examining PFAA concentrations in wild marine mammals also found similar relationships with PFOS concentrations. PFAA concentrations in bottlenose dolphin populations have been shown to decrease with age, with males also having higher concentrations than females (Fair et al., 2012). Sex-based differences may result from maternal offloading of contaminants in milk or eggs, which has been reported for marine mammals and bird species (Fair et al., 2012; Newsted et al., 2007). However, PFAA excretion rates are extremely species-specific (Chang et al., 2012), and PFAA bioaccumulation patterns in reptiles need further examination. A study on wild Nile crocodiles found no significant relationship between PFAA concentration and length or sex, similar to this study (Christie et al., 2016). In contrast to body size and sex, the growth and feeding patterns of American alligators (and crocodilians in general) complicate the potential for uncovering a relationship between PFAA concentrations and age, as comparing concentrations in alligators of similar length, but with potentially varied age and diet obscures potential relationships between contaminants they may have bioaccumulated over their lifetimes. Alligators are a long-lived species with determinate growth (Wilkinson et al., 2016; Wilkinson and Rhodes, 1997). That is, they may continue growth for a period after reaching reproductive maturity, but growth stops well before reaching senescence (Wilkinson et al., 2016). As a result, although several animals in a given population might be the same size (TL), they may be very different in age. As such, body size (*e.g.*, length) of alligators, especially adults, cannot reliably be used as a proxy for age. Ontogenetic and site-specific differences in alligator diet may also influence exposure of alligators to PFAAs (Smith et al., 2007).

Site-specific PFAA accumulation has been well documented in wildlife from around the world (Berger et al., 2009; Christie et al., 2016; Persson and Magnusson, 2015). Tail muscle samples from alligators harvested from two specific hunt units in SC, the Southern Coastal and Middle Coastal units, were used to examine spatial variation in PFAA concentrations. The two other hunt units, Midlands and Pee Dee, were not included in these comparisons due to small samples sizes from these areas ($n = 5$ and $n = 2$, respectively). This study found a significant relationship between PFAA concentrations and the location of harvest, with samples from the Middle Coastal hunt unit having significantly higher concentrations of PFDA ($p = 0.01$), PFUnA ($p = 0.0039$), PFDoA ($p = 0.0002$), PFTrIA ($p = 0.0003$), and

PFOS ($p = 0.0001$) than those from the Southern Coastal hunt unit (Fig. 2). Median PFOS concentrations (Table 1) in the Middle Coastal and Midlands hunt units were elevated (16.0 and 13.1 ng/g, respectively) compared to the Southern Coastal hunt unit (3.01 ng/g). The Midlands hunt unit was also found to have comparable median concentration levels to the Middle Coastal unit for PFDA, PFUnA, PFDoA and PFTrIA (Table 1). The two hunt units with the highest PFAA concentrations, the Middle Coastal and Midlands, are roughly adjacent to one another and fall within the same watershed, suggesting a potential shared PFAA source (Fig. 1).

Comparison between individual water body of harvest (within hunt units) and PFAA concentrations were also examined to explore potential local sources of contaminant exposure. Charleston Harbor is centrally located in the Middle Coastal hunt unit and has been described as a hotspot for PFAA contamination in previous wildlife and sediment studies (Fair et al., 2012; White et al., 2015). Both studies found PFOS to be the dominant compound in samples, similar to this study. Despite the phase-out of many PFAAs used by industry, the elevated PFAA concentrations in the Charleston Harbor area could indicate that sources of contamination to this watershed are potentially active, there is transport and accumulation of PFAAs in this area from an upstream location, and/or there is accumulation of PFAAs in a closed system.

The Cooper River runs through Berkley County, SC receiving water from Lake Moultrie within the Midlands unit, and flowing toward the coast into Charleston Harbor. When alligator harvest locations were separated by county for further site-specific investigation, Berkley County within the Middle Coastal hunt unit exhibited the highest PFDA, PFTrIA, and PFOS concentrations of the four counties included in the study. Four out of the five samples with the highest PFOS concentrations were from Berkley County (Supplemental information, Table S1), with a maximum PFOS concentration of 36.4 ng/g in an alligator harvested from the Cooper River. Both Beaufort and Colleton Counties, within the Southern Coastal hunt unit, were found to have significantly lower PFOS concentrations than Berkley County ($p < 0.0001$). These data illustrate the site-specific nature of PFAA exposure, with the highest concentrations detected from hunt units within the same watershed and supporting previous studies demonstrating Charleston Harbor as a hotspot for PFAA contamination. This study also provides evidence that Charleston Harbor may be receiving PFAAs from potential inputs higher (upstream) in the watershed such as the Cooper River, indicated by the highest concentrations of PFAAs in samples from this waterbody. Many potential sources for PFAAs exist along the Cooper River, including industry, development, a naval station, and wastewater treatment outflows, all of which have been previously identified as sources of PFAA pollution (Adams et al., 2008; Arvaniti and Stasinakis, 2015; Gallen et al., 2014).

The lack of species-specific advisories leave SC wild game consumers to rely on fish consumption guidelines set by other states and European countries. No samples examined in this study had PFOS concentrations exceeding the no restrictions limit for fish of 40 ng/g set by the Minnesota Department of Health (Minnesota Department of Health, 2008). However, there are many waterbodies within Minnesota for which their Department of Health recommends fish consumption of no more than one meal per month for women who

are or may become pregnant and children under the age of 15 (Minnesota Department of Health, 2016). The maximum PFOS concentrations found in the present study (36.4 ng/g) was not far from reaching the general Minnesota consumption threshold based on one meal per week (from 40 to 200 ng/g). The general consumption advisory, based on PFOS levels found in fish, does not describe consumption rates, meal sizes or provide a guideline specific to vulnerable populations. Although the health effects from chronic PFOS exposure are not yet fully understood, the U.S. Environmental Protection Agency recently decreased the drinking water advisory (for combined PFOS and PFOA) from 400 to 70 pg/mL based on new evidence of long term exposure health effects (*e.g.*, developmental abnormalities, cancers, liver and thyroid disorders) found in laboratory and human studies (EPA, 2016). Consumption advisories for PFOS also do not take into account the potential health effects from mixtures of PFAAs or total PFAAs combined. Overall, this study illustrates the need for further studies on PFAA accumulation in wildlife, particularly game species, and the potential effects of these chemicals on wildlife and human health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to acknowledge Jackie Bangma and Thomas Galligan for their assistance with sample collection. We would like to give special thanks to Cordray's Processing and Taxidermy for allowing us to utilize their facility and to collect samples. Support for this research was provided partially by the Graduate School at the College of Charleston. This paper represents Technical Contribution No. 6542 of the Clemson University Experiment Station.

REFERENCES

- Adams J, Houde M, Muir D, Speakman T, Bossart G, Fair P, 2008 Land use and the spatial distribution of perfluoroalkyl compounds as measured in the plasma of bottlenose dolphins (*Tursiops truncatus*). *Mar. Environ. Res* 66, 430–437. [PubMed: 18768218]
- Ahrens L, Siebert U, Ebinghaus R, 2009 Total body burden and tissue distribution of polyfluorinated compounds in harbor seals (*Phoca vitulina*) from the German Bight. *Mar. Pollut. Bull* 58, 520–525. [PubMed: 19121527]
- Allsteadt J, Lang JW, 1995 Sexual dimorphism in the genital morphology of young American alligators, *Alligator mississippiensis*. *Herpetologica* 51, 314–325.
- Armitage J, Cousins IT, Buck RC, Prevedouros K, Russell MH, MacLeod M, et al., 2006 Modeling global-scale fate and transport of perfluorooctanoate emitted from direct sources. *Environ. Sci. Technol* 40, 6969–6975. [PubMed: 17154003]
- Armitage JM, MacLeod M, Cousins IT, 2009 Modeling the global fate and transport of perfluorooctanoic acid (PFOA) and perfluorooctanoate (PFO) emitted from direct sources using a multispecies mass balance model. *Environ. Sci. Technol* 43, 1134–1140. [PubMed: 19320170]
- Arvaniti OS, Stasinakis AS, 2015 Review on the occurrence, fate and removal of perfluorinated compounds during wastewater treatment. *Sci. Total Environ* 524–525, 81–92.
- Bangma JT, Bowden JA, Brunell AM, Christie I, Finnell B, Guillet MP, et al., 2017a Perfluorinated alkyl acids in plasma of American alligators (*Alligator mississippiensis*) from Florida and South Carolina. *Environ. Toxicol. Chem* 36, 917–925. [PubMed: 27543836]
- Bangma JT, Reiner JL, Jones M, Lowers RH, Nilsen F, Rainwater TR, et al., 2017b Variation in perfluoroalkyl acids in the American alligator (*Alligator mississippiensis*) at Merritt Island National Wildlife Refuge. *Chemosphere* 116, 72–79.

- Berger U, Glynn A, Holmstrom KE, Berglund M, Ankarberg EH, Tornkvist A, 2009 Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden – analysis of edible fish from Lake Vattern and the Baltic Sea. *Chemosphere* 76, 799–804. [PubMed: 19457539]
- Butfiloski J, 2014 Alligator Hunting Season Report 2013 South Carolina Department of Natural Resources, Wildlife and Freshwater Fisheries Division (F&AP Report).
- Butfiloski J, 2015 Alligator Hunting Season Report 2015 South Carolina Department of Natural Resources, Wildlife and Freshwater Fisheries Division (F&AP Report).
- Butt CM, Mabury SA, Kwan M, Wang XW, Muir DCG, 2008 Spatial trends of perfluoroalkyl compounds in ringed seals (*Phoca hispida*) from the Canadian Arctic. *Environ. Toxicol. Chem* 27, 542–553. [PubMed: 17988182]
- Butt CM, Berger U, Bossi R, Tomy GT, 2010 Levels and trends of poly- and perfluorinated compounds in the arctic environment. *Sci. Total Environ* 408, 2936–2965. [PubMed: 20493516]
- Chang SC, Noker PE, Gorman GS, Gibson SJ, Hart JA, Ehresman DJ, et al., 2012 Comparative pharmacokinetics of perfluorooctanesulfonate (PFOS) in rats, mice, and monkeys. *Reprod. Toxicol* 33, 428–440. [PubMed: 21889587]
- Christie I, Reiner JL, Bowden JA, Botha H, Cantu TM, Govender D, et al., 2016 Perfluorinated alkyl acids in the plasma of South African crocodiles (*Crocodylus niloticus*). *Chemosphere* 154, 72–78. [PubMed: 27038902]
- EPA, 2000 In: Register F (Ed.), Perfluorooctyl Sulfonates: Proposed Significant New Use Rule 65, pp. 69889–69890.
- EPA, 2016 Fact sheet: PFOA and PFOS drinking water health advisories <https://www.epa.gov/ground-water-and-drinking-water/drinking-water-health-advisories-pfoa-and-pfos> (Accessed January 11, 2017).
- Fair PA, Houde M, Hulsey TC, Bossart GD, Adams J, Balthis L, et al., 2012 Assessment of perfluorinated compounds (PFCs) in plasma of bottlenose dolphins from two southeast US estuarine areas: relationship with age, sex and geographic locations. *Mar. Pollut. Bull* 64, 66–74. [PubMed: 22118898]
- Fair PA, Romano T, Schaefer AM, Reif JS, Bossart GD, Houde M, et al., 2013 Associations between perfluoroalkyl compounds and immune and clinical chemistry parameters in highly exposed bottlenose dolphins (*Tursiops truncatus*). *Environ. Toxicol. Chem* 32, 736–746. [PubMed: 23322558]
- Gallen C, Baduel C, Lai FY, Thompson K, Thompson J, Warne M, et al., 2014 Spatio-temporal assessment of perfluorinated compounds in the Brisbane River system, Australia: impact of a major flood event. *Mar. Pollut. Bull* 85, 597–605. [PubMed: 24613231]
- Gannon SA, Johnson T, Nabb DL, Serex TL, Buck RC, Loveless SE, 2011 Absorption, distribution, metabolism, and excretion of [1–14C]-perfluorohexanoate ([14C]-PFHx) in rats and mice. *Toxicology* 283, 55–62. [PubMed: 21349313]
- Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, et al., 2012 Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *J. Am. Med. Assoc* 307, 391–397.
- Guruge KS, Hikono H, Shimada N, Murakami K, Hasegawa J, Yeung LW, et al., 2009 Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F1 mice. *J. Toxicol. Sci* 34, 687–691. [PubMed: 19952504]
- Jantzen CE, Annunziato KA, Bugel SM, Cooper KR, 2016 PFOS, PFNA, and PFOA sub-lethal exposure to embryonic zebrafish have different toxicity profiles in terms of morphometrics, behavior and gene expression. *Aquat. Toxicol* 175, 160–170. [PubMed: 27058923]
- Keller JM, Ngai L, Braun McNeill J, Wood LD, Stewart KR, O'Connell SG, et al., 2012 Perfluoroalkyl contaminants in plasma of five sea turtle species: comparisons in concentration and potential health risks. *Environ. Toxicol. Chem* 31, 1223–1230. [PubMed: 22447337]
- Kim M, Son J, Park MS, Ji Y, Chae S, Jun C, et al., 2013 In vivo evaluation and comparison of developmental toxicity and teratogenicity of perfluoroalkyl compounds using *Xenopus* embryos. *Chemosphere* 93, 1153–1160. [PubMed: 23910242]
- Kjeldsen LS, Bonefeld-Jørgensen EC, 2013 Perfluorinated compounds affect the function of sex hormone receptors. *Environ. Sci. Pollut. Res* 20, 8031–8044.

- Kudo N, Suzuki E, Katakura M, Ohmori K, Noshiro R, Kawashima Y, 2001 Comparison of the elimination between perfluorinated fatty acids with different carbon chain length in rats. *Chem. Biol. Interact* 134, 203–216. [PubMed: 11311214]
- Lindstrom AB, Strynar MJ, Libelo EL, 2011 Polyfluorinated compounds: past, present, and future. *Environ. Sci. Technol* 45, 7954–7961. [PubMed: 21866930]
- Loi EI, Yeung LWY, Taniyasu S, Lam PKS, Kannan K, Yamashita N, 2011 Trophic magnification of poly- and perfluorinated compounds in a subtropical food web. *Environ. Sci. Technol* 45, 5506–5513. [PubMed: 21644538]
- Milnes MR, Guillette LJ, 2008 Alligator tales: new lessons about environmental contaminants from a sentinel species. *Bioscience* 58, 1027–1036.
- Minnesota Department of Health, 2008 Fish consumption advisory program: meal advice categories based on PFOS in fish <http://www.health.state.mn.us/divs/eh/fish/eating/mealadvicetables.pdf> (Accessed January 11, 2017).
- Minnesota Department of Health, 2016 Fish consumption guidelines for women who are or may become pregnant and children under age 15 <http://www.health.state.mn.us/divs/eh/fish/eating/specpoprivers.pdf> (Accesses January 11, 2017).
- Muller CE, De Silva AO, Small J, Williamson M, Wang X, Morris A, et al., 2011 Biomagnification of perfluorinated compounds in a remote terrestrial food chain: Lichen–Caribou–Wolf. *Environ. Sci. Technol* 45, 8665–8673. [PubMed: 21905660]
- Newsted JL, Coady KK, Beach SA, Butenhoff JL, Gallagher S, Giesy JP, 2007 Effects of perfluorooctane sulfonate on mallard and northern bobwhite quail exposed chronically via the diet. *Environ. Toxicol. Pharmacol* 23, 1–9. [PubMed: 21783730]
- Nifong JC, Silliman BR, 2013 Impacts of a large-bodied, apex predator (Alligator mississippiensis Daudin 1801) on salt marsh food webs. *J. Exp. Mar. Biol. Ecol* 440, 185–191.
- Olsen GW, Lange CC, Ellefson ME, Mair DC, Church TR, Goldberg CL, et al., 2012 Temporal trends of perfluoroalkyl concentrations in American Red Cross adult blood donors, 2000–2010. *Environ. Sci. Technol* 46, 6330–6338. [PubMed: 22554481]
- Persson S, Magnusson U, 2015 Environmental pollutants and alterations in the reproductive system in wild male mink (*Neovison vison*) from Sweden. *Chemosphere* 120, 237–245. [PubMed: 25103085]
- Platt SG, Rainwater TR, Thorbjarnarson JB, Finger AG, Anderson TA, McMurry ST, 2009 Size estimation, morphometrics, sex ratio, sexual size dimorphism, and biomass of Morelet's crocodile in northern Belize. *Caribb. J. Sci* 45, 80–93.
- Platt SG, Rainwater TR, Thorbjarnarson JB, Martin D, 2011 Size estimation, morphometrics, sex ratio, sexual size dimorphism, and biomass of *Crocodylus acutus* in the Coastal Zone of Belize. *Salamandra* 47, 179–192.
- Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH, 2006 Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol* 40, 32–44. [PubMed: 16433330]
- Reiner JL, O'Connell SG, Butt CM, Mabury SA, Small JM, De Silva AO, et al., 2012 Determination of perfluorinated alkyl acid concentrations in biological standard reference materials. *Anal. Bioanal. Chem* 404, 2683–2692. [PubMed: 22476786]
- Smith PN, Cobb GP, Godard-Codding C, Hoff D, McMurry ST, Rainwater TR, et al., 2007 Contaminant exposure in terrestrial vertebrates. *Environ. Pollut* 150, 41–64. [PubMed: 17706848]
- Wang J, Zhang Y, Zhang F, Yeung LWY, Taniyasu S, Yamazaki E, et al., 2013 Age- and gender-related accumulation of perfluoroalkyl substances in captive Chinese alligators (*Alligator sinensis*). *Environ. Pollut* 179, 61–67. [PubMed: 23660395]
- White ND, Balthis L, Kannan K, De Silva AO, Wu Q, French KM, et al., 2015 Elevated levels of perfluoroalkyl substances in estuarine sediments of Charleston, SC. *Sci. Total Environ* 521–522, 79–89.
- Wilkinson PM, Rhodes WE, 1997 Growth rates of American alligators in coastal south Carolina. *J. Wildl. Manag* 61, 397–402.
- Wilkinson PM, Rainwater TR, Woodward AR, Leone EH, Carter C, 2016 Determinate growth and reproductive lifespan in the American alligator (*Alligator mississippiensis*): evidence from long-term recaptures. *Copeia* 104, 843–852.

- Yamashita N, Taniyasu S, Petrick G, Wei S, Gamo T, Lam PK, et al., 2008 Perfluorinated acids as novel chemical tracers of global circulation of ocean waters. *Chemosphere* 70, 1247–1255. [PubMed: 17854858]
- Yordy JE, Rossman S, Ostrom PH, Reiner JL, Bargnesi K, Hughes S, et al., 2013 Levels of chlorinated, brominated, and perfluorinated contaminants in birds of prey spanning multiple trophic levels. *J. Wildl. Dis* 49, 347–354. [PubMed: 23568910]
- Zhang W, Sheng N, Wang M, Zhang H, Dai J, 2016 Zebrafish reproductive toxicity induced by chronic perfluorononanoate exposure. *Aquat. Toxicol* 175, 269–276. [PubMed: 27082981]

Fig. 1.
Map of South Carolina showing the location of American alligator (*Alligator mississippiensis*) public hunt units.

Fig. 2. Mean PFDA, PFUnA, PFDoA, and PFOS concentrations in American alligator (*Alligator mississippiensis*) tail muscle samples collected from the Southern Coastal ($n = 19$) and Middle Coastal ($n = 17$) hunt units during the 2015 South Carolina public hunt. Error bars represent the standard deviation. * indicates statistical significance ($p < 0.05$).

PFNA concentrations (ng/g wet mass) in tail muscle samples from American alligators (*Alligator mississippiensis*) harvested in 2015 during the South Carolina public hunt.

Table 1 –

	Hunt unit 1 — Southern Coastal			Hunt unit 2 — Middle Coastal			Hunt unit 3 — Midlands			Hunt unit 4 — Pee Dee		
	Tail muscle tissue (n = 19)			Tail muscle tissue (n = 17)			Tail muscle tissue (n = 5)			Tail muscle tissue (n = 2)		
	Range	Median	n > RL *	Range	Median	n > RL *	Range	Median	n > RL *	Range	Median	n > RL *
PFNA	<0.088–0.551	0.107	14	<0.073–0.553	0.102	11	NA	NA	0	0.100–0.135	0.117	2
PFDA	0.182–3.09	0.556	19	0.172–4.06	2.21	17	1.14–2.93	1.74	5	0.716–0.990	0.853	2
PFUnA	0.194–5.32	0.504	19	0.228–9.27	3.77	17	2.58–6.87	5.71	5	0.725–1.61	1.17	2
PFDoA	0.111–3.72	0.248	19	0.143–9.09	2.99	17	2.31–6.97	5.03	5	0.262–1.63	0.946	2
PFTriA	<0.063–0.785	0.164	18	0.102–2.58	0.789	17	0.592–2.37	1.36	5	0.115–0.456	0.286	2
PFTA	<0.07–1.57	0.050	8	<0.07–1.32	0.492	13	0.381–1.6	0.834	5	<0.07–0.131	NA	1
PFHxS	0.051–0.252	0.087	19	0.063–0.272	0.099	17	0.054–0.158	0.0816	5	0.071–0.115	0.093	2
PFOS	1.02–17.3	3.01	19	3.24–36.4	16.0	17	8.29–19.9	13.1	5	5.78–7.35	6.56	2
PFOSA	<0.056–1.43	NA	4	<0.061–1.06	0.482	14	0.087–0.560	NA	5	<0.068–0.193	NA	1

NA: not applicable; PFNA: perfluorinated alkyl acid; PFNA: perfluorononanoic acid; PFDA: perfluorodecanoic acid; PFUnA: perfluoroundecanoic acid; PFDoA: perfluorododecanoic acid; PFTriA: perfluorotridecanoic acid; PFTA: perfluorotetradecanoic acid; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctane sulfonate; PFOSA: perfluorooctanesulfonamide.

* n > RL indicates the number of samples above the reporting limit (RL).