Age- and gender-related accumulation of perfluoroalkyl substances in captive Chinese alligators (*Alligator sinensis*)

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ABSTRACT

Fourteen perfluoroalkyl substances (PFASs) were measured in serum of the highly endangered captive Chinese alligators, whole body homogenates of six kinds of fish (alligator prey species), and pond water (alligator habitat) in the Anhui Research Center for Chinese Alligator Reproduction. Six PFASs, including PFOS and five perfluorinated carboxylates, were detected in all alligator samples. The most dominant PFAS was PFUnDA, with a mean value of 31.4 ng/mL. Significant positive correlations were observed among the six PFASs, suggesting that they shared similar sources of contamination. Significantly higher PFOS and PFUnDA levels were observed in males, but the other four PFCAs did not differ between genders. An age related PFAS bioaccumulation analysis showed a significant negative correlation of the concentrations for five PFCAs to age, which means that higher concentrations were found in younger animals. Bioaccumulation factors (BAF) in fish for PFASs ranged from 21 to 28,000, with lower BAF for PFOA than that for longer carbon chain PFCAs, including PFUnDA, PFDA, and PFNA.

1. Introduction

Poly-/perfluoroalkyl substances (PFASs), which have been manufactured for more than 50 years, are comprised of a diverse group of chemicals used in a variety of specialized consumer and industrial products, such as surfactants and surface protectors in textiles, carpets, paper products, fire-fighting foams, food containers, and upholstery (Prevedouros et al., 2006). PFASs include perfluorinated acids and precursors (e.g., polyfluorinated telomer alcohols, sulfonamides); the former one can be further divided into two main groups: perfluoroalkane sulfonic acids (PFSAs) and perfluorinated carboxylic acids (PFCAs). Given the high energy of the carbon–fluorine bond, PFASs are resistant to hydrolysis, photolysis, biodegradation, and metabolism. These compounds have been found on a global scale in air, water, and house dust (Boulanger et al., 2005; Giesy and Kannan, 2002; Hansen et al., 2002; Harada et al., 2006; Shoeib et al., 2005; So et al., 2004); they have also been reported in

wildlife species including marine animals, sea birds, fish, and terrestrial mammals (Giesy and Kannan, 2001; Kannan et al., 2002a, 2002b, 2002c; Martin et al., 2003a, 2004; Smithwick et al., 2005). Among the PFASs analyzed in different environmental matrices, perfluorooctane sulfonate (PFOS) has been frequently found at the highest concentrations (Giesy and Kannan, 2001; Kannan et al., 2001, 2002a, 2002b, 2002c; Martin et al., 2004; Moody et al., 2002); other PFASs, such as perfluorohexane sulfonate (PFHxS), perfluorododecanoic acid (PFDoDA), perfluoroundecanoic acid (PFUnDA), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), and perfluorooctanoic acid (PFOA) have been detected at an order of magnitude lower than that of PFOS in many biological samples (Houde et al., 2006b). Studies have suggested that PFSAs are more bioaccumulative than PFCAs having the same fluorinated carbon chain length; PFCAs with fluorinated carbon chain length greater than seven are bioaccumulative, and the biomagnification factors increase with the number of the fluorinated carbon (Conder et al., 2008; Kannan, 2011). Animal exposure studies have suggested that PFASs (e.g., PFOA) can cause various toxic effects, including hepatotoxicity, developmental toxicity, immunotoxicity and hormonal effects (Lau et al., 2007; Peters and Gonzalez, 2011). Although the effect on health from long-term environmental PFAS exposure is

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still relatively unknown, given the ubiquitous presence and persistence of these chemicals and the toxicity observed in animal studies, these environmental contaminants might present a health risk to wildlife.

Fluorotelomer alcohols (FTOHs) are an indirect source of PFCAs; they are emitted through industrial activities and can form PFASs upon degradation (Ellis et al., 2004). For this reason, animals residing in environments close to (or in the vicinity of) industrial hotspots might accumulate high levels of PFASs; the Chinese alligator (*Alligator sinensis*) may be one such example. The Chinese alligator is one of the most endangered species among the world's twenty-three crocodilian species (IUCN Red List: CR A1c, D (critically endangered)). Historically, these animals were distributed across relatively wide areas around the lower Yangtze River in China. Habitat destruction is one of the major causes for their decline, particularly the transformation of many wetland areas into agricultural land. Deliberate killing of alligators also results in significant negative effects. In addition, several studies have shown that environmental factors, such as the application of chemical fertilizers and insecticides, might affect the health of the Chinese alligators (Thorbjarnarson and Wang, 1999). The remaining wild individuals are now restricted to a few small isolated areas in Anhui and Zhejiang Provinces (Thorbjarnarson et al., 2002); the total wild population is believed to be less than 200. Fortunately, breeding and growth in captivity has been occurring successfully since 1979 at the Anhui Research Center for Chinese Alligator Reproduction (ARCCAR), Xuancheng City, Anhui Province; the captive population is now over 10,000 animals. With rapid economic development, China is facing increasing problems with a variety of environmental contaminants including PFASs (Bao et al., 2010). Captive animals residing near urban industrialized areas might have increased exposures to the contaminants, possibly adversely affecting their health.

The objectives of the current study were: a) to assess exposure of PFASs in Chinese alligators by measuring the concentrations of PFASs in alligators from a conservation center situated in an urbanized region of China; b) to evaluate any gender- and age-specific PFASs accumulation; and c) to explore the sources of PFAS contamination.

2. Materials and methods

2.1. Sample collection

Sera samples (3–5 mL per animal) were collected by a veterinarian using sterile syringes and needles from the caudal vein of 48 shallow hibernant crocodiles in ARCCAR in November 2009 (Supporting Information (SI) Fig. S1). For serum isolation, samples were allowed to sit at room temperature for 30 min to allow the blood to clot. Separation of clots was accomplished by centrifugation at 1000–1300 g at 4 °C for 15–20 min. The serum was removed and dispensed in polypropylene cryovials aliquots of 1 mL. The alligators with different age stages (2–9 years, 10–15 years, and 16–21 years) were raised in divided areas in the ARCCAR, though the exact age for each alligator was not available. The gender, age groups, weight, and length of the animals were recorded (Table 1). The blood collection protocol was approved by the Institute of Zoology, Chinese Academy of Sciences, Institutional Animal Care Committee and State Forestry Administration of China. Silver carp (*Hypophthalmichthys molitrix*), oriental river prawn (*Macrobrachium nipponense*), northern snakehead fish (*Channa argus*), tire track eel (*Mastacembelus armatus*), crucian carp (*Carassius carassius*), and common carp (*Cyprinus carpio*), which all

serve as part of the alligator's diet, were collected from the habitat pond of the alligators. In addition, water (2 × 4 L) from the pond was sampled at the same time. All samples were kept at −20 °C until analysis.

2.2. Perfluoroalkyl substance analysis

Thawed alligator serum, water, and homogenized whole fish and prawn samples were analyzed for fourteen PFASs; four PFSAs: perfluorodecane sulfonate (PFDS), PFOS, PFHxS, and perfluorobutane sulfonate (PFBS); and ten PFCAs: perfluorotetradecanoic acid (PFTeDA), PFDoDA, PFUnDA, PFDA, PFNA, PFOA, perfluoroheptanoic acid (PFHpA), perfluorohexanoic acid (PFHxA), perfluoropentanoic acid (PFPeA), and perfluorobutanoic acid (PFBA). Before extraction, 1 mL sera samples were spiked with an internal standard mixture ([$^{13}C_4$]-PFOA, [$^{13}C_5$]-PFNA, [$^{13}C_2$]-PFDA, [$^{13}C_4$]-PFOS and [$^{13}C_4$]-PFBA) to check an overall recovery. Extraction of PFASs from sera samples was achieved using acetonitrile, and the extract was subjected to further purification using the SPE-Oasis-WAX-method (details in SI Information). Final extracts were analyzed using an Agilent HP1100 liquid chromatography system interfaced with a Micromass (Beverly, MA) Quattro Ultima Pt mass spectrometer, which was operated in electrospray negative ionization mode as previously reported (Taniyasu et al., 2005). In brief, a 10 μL aliquot of the sample extract was injected onto a guard column (XDB-C8, 2.1 mm i.d. × 12.5 mm, 5 μm; Agilent Technologies, Palo Alto, CA) connected to a Betasil C18 column (2.1 mm i.d. × 50 mm length, 5 μm, end-capped; Thermo Hypersil-Keystone, Bellefonte, PA). The mobile phases consisted of 2 mM ammonium acetate and methanol, with 10% methanol serving as the starting mobile phase.

2.3. Quality assurance and quality control

Data quality assurance and quality control measures included instrumental blanks, procedural (method) blanks, matrix spikes, and duplicated analysis. Procedural blanks (using Milli-Q water as a matrix) and recoveries (Milli-Q water spiked with native standards) were assessed following the same procedure as described above with each group of extractions. The matrix spike recoveries for native compounds ranged from 60 to 108%. More detailed information for the values of LOQs, instrumental blanks, cartridge blanks, procedural blanks, procedural recoveries, and matrix spike recoveries, as well as MS parameters, are given in SI Table S1. The limits of quantification (LOQs) were 0.01 ng/mL for the all PFASs except PFBA (0.05 ng/mL). The blanks were all below corresponding LOQs. An external calibration curve was prepared from a series of PFAS concentrations (10, 50, 200, 1000, 5000 and 20,000 pg/mL), and standard deviations of the measured values to the theoretical values were less than 20%. Method LOQs were determined by spiking 0.01, 0.05, and 0.1 ng into the test sera samples, the spiked samples went through the whole analytical procedures. This method LOQs experiment was performed in triplicate. The method LOQs (S/N > 10) were found to be 0.01 ng/mL for PFOS, PFHxS, and PFOA; 0.05 ng/mL for PFHpA, PFNA, PFDA, PFUnDA, and PFDoDA; and 0.1 ng/mL for PFTeDA. The recoveries of these compounds between before spike/post spike were between 73 and 95%, and the relative standard deviations were between 5 and 12%. The concentrations of PFASs in the experimental samples were not corrected for recoveries.

2.4. Statistical analysis, bioaccumulation factor, and biomagnification factor calculation

The normality of the data was analyzed using a Shapiro–Wilk test. Mann–Whitney U or Kruskal–Wallis non-parametric tests (two-tailed) were used to compare PFAS concentrations between genders or among different age stages. Spearman's rho rank-order correlation analysis was performed to examine the relationship between PFASs. Linear regression was used to evaluate relationships between body length (used as a proxy for age) and natural log-transformed values for PFAS concentrations. A two-way ANOVA was used to investigate the relationship of PFASs between genders and age. All statistical analyses were conducted with SPSS software (Version 14.0 for windows, SPSS Incorporate, Chicago, IL). The bioaccumulation factors (BAFs) of PFASs were calculated for fish and prawns by dividing their PFAS concentration with the concentration of water. The biomagnification factors (BMFs) were calculated for the alligator relative to each of its prey items by dividing mean alligator sera concentration with mean concentration of the whole fish or prawn homogenates.

3. Results and discussion

3.1. PFAS levels in the Chinese alligators

The sera concentrations of six PFASs (PFOS, PFTeDA, PFDoDA, PFUnDA, PFDA, and PFNA) were all above the LOQ; while another six PFASs (PFHxS, PFBS, PFOA, PFHpA, PFPeA, and PFBA) were detected in alligator sera samples with a detection frequency of 52%, 21%, 69%, 17%, 31%, and 29%, respectively. Detailed statistical

Table 1
Demographic information of the Chinese alligator samples.

Gender	Age	Number	Weight (kg)	Length (cm)
Female	2–9	14	6.6 ± 0.9	123.4 ± 6.9
	10–15	6	13.0 ± 1.9	151.0 ± 7.5
	16–21	6	18.8 ± 2.1	164.2 ± 5.1
Male	2–9	9	6.5 ± 2.3	123.3 ± 12.7
	10–15	7	17.6 ± 1.4	168.0 ± 5.3
	16–21	6	26.1 ± 7.4	184.0 ± 15.4

Table 2
Summary of frequency of detection (%), PFAS concentrations (ng/mL), and percentage of PFAS to total PFAS (%) in serum of Chinese alligators ($n = 48$).

	Frequency of detection (%)	Mean	Std. deviation	Geometric mean	Range	Percentage in total PFASs (%)
PFOS	100	28.7	10.1	26.7	6.7–61.8	30.9
PFHxS	52	0.2	0.3	0.0	0–1.5	0.2
PFBS	21	0.004	0.009	0.0	0–0.04	0.004
PFTeDA	100	1.1	1.6	0.4	0.1–6.2	1.2
PFDoDA	100	2.7	1	2.5	0.8–5.9	2.9
PFUnDA	100	31.4	11.7	28.8	7.8–63.0	33.8
PFDA	100	26	11.1	23.1	4.7–56.0	27.9
PFNA	100	2.9	2.7	2.2	0.2–18.8	3.1
PFOA	69	0.02	0.02	0.0	0–0.1	0.02
PFHpA	17	0.003	0.007	0	0–0.03	0.003
PFPeA	31	0.005	0.01	0	0–0.1	0.006
PFBA	29	0.008	0.01	0	0–0.1	0.009

Samples with concentration below LOQ were treated as zero.

information of these twelve PFASs is given in Table 2. No detectable PFDS or PFHxA were found in any of the sera samples. The concentrations of PFOS, PFTeDA, PFDoDA, PFUnDA, PFDA, and PFNA ranged from 0.1 to 63.0 ng/mL. Results showed that PFUnDA (mean 31.4 ng/mL) had the greatest concentrations among the PFASs; it ranged from 7.8 to 63.0 ng/mL, and accounted for 33.8% of total PFASs for all twelve PFASs. The second and third dominant PFASs were PFOS (28.7 ng/mL) and PFDA (26 ng/mL), accounting for 30.9% and 27.9% of the total PFASs, respectively. In decreasing order of abundance were PFNA (2.9 ng/mL), PFDoDA (2.7 ng/mL), and PFTeDA (1.1 ng/mL), which were an order of magnitude lower than the concentrations of PFUnDA, PFOS, and PFDA; these compounds contributed 7.2% to the total PFASs.

Sera PFOS concentrations in the alligators of this study were in a similar range to those found in other reptiles from other locations, such as Kemp's ridley sea turtles (*Lepidochelys kempii*) and Loggerhead sea turtles (*Caretta caretta*) from the southeastern coast of USA (Keller et al., 2005). Keller's study has shown that PFOS was the most dominant PFAS in the two kinds of turtles, with concentration an order of magnitude higher than those of other PFASs. However, in the present study, PFUnDA was the dominant PFAS. Although PFUnDA has been reported in biota (Guruge et al., 2005; Hoff et al., 2004), few studies (Muir et al., 2004) have reported PFUnDA as being the most dominant PFAS in wildlife samples. Concentrations of PFDA and PFOS were in our study in the same order of magnitude. PFUnDA and PFDA contributed 60.3% to total PFASs. Some studies have shown that PFNA was the dominant PFCA in the livers of polar bears and Amur tigers (Li et al., 2008; Smithwick et al., 2005), however, in the present study, PFNA contributed to only

3.8% to the total PFASs. Several studies have shown detectable PFOA concentrations in wildlife and human sera samples (Kannan et al., 2004, 2005b). In the present study, only 69% of sera samples showed detectable PFOA concentrations, with the greatest level at only 0.1 ng/mL. Short-carbon chain PFCAs, such as PFPeA, PFBA, and PFHpA, only showed detectable concentrations in 31%, 29%, and 17% of the sera samples, respectively; PFHxA was not detected in any sample. PFASs with fluorinated carbon numbers greater than seven are thought to be bioaccumulative; while those having less than seven fluorinated carbons are less bioaccumulative and therefore readily excreted (Martin et al., 2003a, 2003b). The results of the present study: relatively high concentrations of long-chain PFCAs (C9-12, and C14) and relatively low concentrations of short-chain PFCAs (C4-7), were consistent with this assumption.

3.2. Gender- and age-related PFAS bioaccumulation

The sera PFAS concentrations stratified by gender (26 female and 22 male) and age (2–9, 10–15, and 16–21 years) are given in SI Table S2. Gender- and age-related PFAS bioaccumulation analyses were carried out with two way ANOVA using gender and age as factors, followed by Tukey's test. No interaction was observed between the two factors. Male alligators had significantly higher PFOS ($p = 0.006$) and PFUnDA ($p = 0.03$) concentrations than females, but no significant differences were observed for PFDA, PFDoDA, PFNA, and PFTeDA between gender (Fig. 1A). However, reports on the relationship between PFOS accumulation and gender are inconsistent; some studies have reported no significant differences in PFOS concentrations between genders (Ahrens et al., 2009; Kannan et al., 2002c; Keller et al., 2005; Van de Vijver et al., 2007); higher PFOS concentrations were found in female harbor porpoises (Van de Vijver et al., 2003) but higher concentrations of PFOS were observed in male snapping turtles collected from Michigan (Kannan et al., 2005a).

Age-related PFAS bioaccumulation patterns were found for five PFAS ($p < 0.01$), but not for PFOS (Fig. 1B). Sera samples from younger animals (age: 2–9) had higher concentrations of PFTeDA, PFDoDA, PFUnDA, PFDA, and PFNA. Since PFUnDA concentrations were significantly different between genders and age groups in alligators, age-related bioaccumulation patterns of PFUnDA by gender were additionally shown in SI Fig. S2. Significant decrease in PFUnDA concentrations was observed with the increase in age in both genders ($p < 0.01$).

The age, sex and body-size of wild alligators and their relationships were difficult or even impossible to attain. Studies had observed their relationships in captive animals, and found that body-size measurements, including total body length, were simple and indirect estimators of age, though considerable evidences
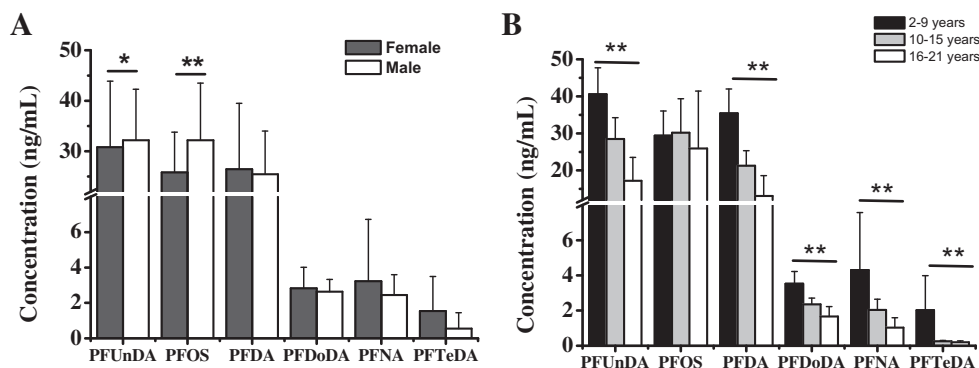

Fig. 1. Graphs showing the relationships of serum PFAS concentrations in subgroups of Chinese alligators by gender (A) and age (B). Data are expressed as mean ± standard derivation. Significant differences between gender or age groups are indicated by * for $p < 0.05$ and ** for $p < 0.01$.

supporting high variation in body size for a given age in crocodilians (Eaton and Link, 2011; Wu et al., 2006). In our study, the age of the alligators at ARCCAR was not accurate, therefore, animal body length was used as a proxy for age, and correlation analysis of body lengths with PFASs was conducted. Among the Crocodylia family, males are larger than females (Fitch, 1981; Platt et al., 2009); the correlation analysis of length with PFASs was performed by gender separately. Significant negative correlations were found between length (age) and the five PFCA concentrations in both male and female alligators, and significant negative correlation between length (age) and PFOS was only found in females (Figs. 2 and 3). Different relationships between age and PFAS levels have been observed in wildlife previously; no significant correlations were observed in gray and ringed seals from the Baltic or Mediterranean Sea (for PFOS) (Kannan et al., 2001), in pandas (for PFOA and PFOS) (Dai et al., 2006), or in Amur tigers (for PFOS and PFNA) (Li et al., 2008); however, increased PFAS concentrations with age have been observed in juvenile male bears (for PFOS, PFNA, and C10–C14 PFCAs) (Smithwick et al., 2005), in Arctic ringed seals (for PFDoDA and PFOS) (Butt et al., 2007), and in ridley turtles (for PFOS) (Keller et al., 2005); contrary, decreased PFAS concentrations with age were found in bottlenose dolphins (Fair et al., 2012; Houde et al., 2005) and juvenile harbor porpoises (Van de Vijver et al., 2003). Although the reason for negative correlations between age and the five PFCA concentrations in alligators was not clear, the somatic growth dilution may be one of the contributors, which had been

reported to occur in methyl-mercury studies in aquatic organisms, such as in zooplankton (Karimi et al., 2007).

These inconsistent results in gender- and age-related PFAS bioaccumulation in wildlife may relate to the complexity of the PFAS bioaccumulation process, and some confounding factors likely contribute to the pattern, e.g., food source. The captive alligators of different age prey on different food, which might have different degrees of PFASs contamination, and thus the observed bioaccumulation pattern might come from different food source in the captive alligators.

3.3. Relationships among PFASs

The results of non-parametric Spearman's rho rank-order correlation analysis could provide further information on the sources of PFASs. Significant positive correlations were found among the six PFASs (SI Table S3). The lowest correlations were observed between PFOS and PFDA (correlation coefficient: $r = 0.44$, $p < 0.01$) and the highest between PFDA and PFDoDA ($r = 0.96$, $p < 0.01$), respectively. These findings suggest that these six PFASs have a similar source of contamination. When the samples were stratified by gender, significant positive correlations (r ranged from 0.66 to 0.96) were observed among all six PFASs in the female subgroup, whereas only five PFASs showed significant positive correlations (r ranged from 0.70 to 0.97) in the male subgroup; no significant correlations were found between PFOS and the other five PFASs in
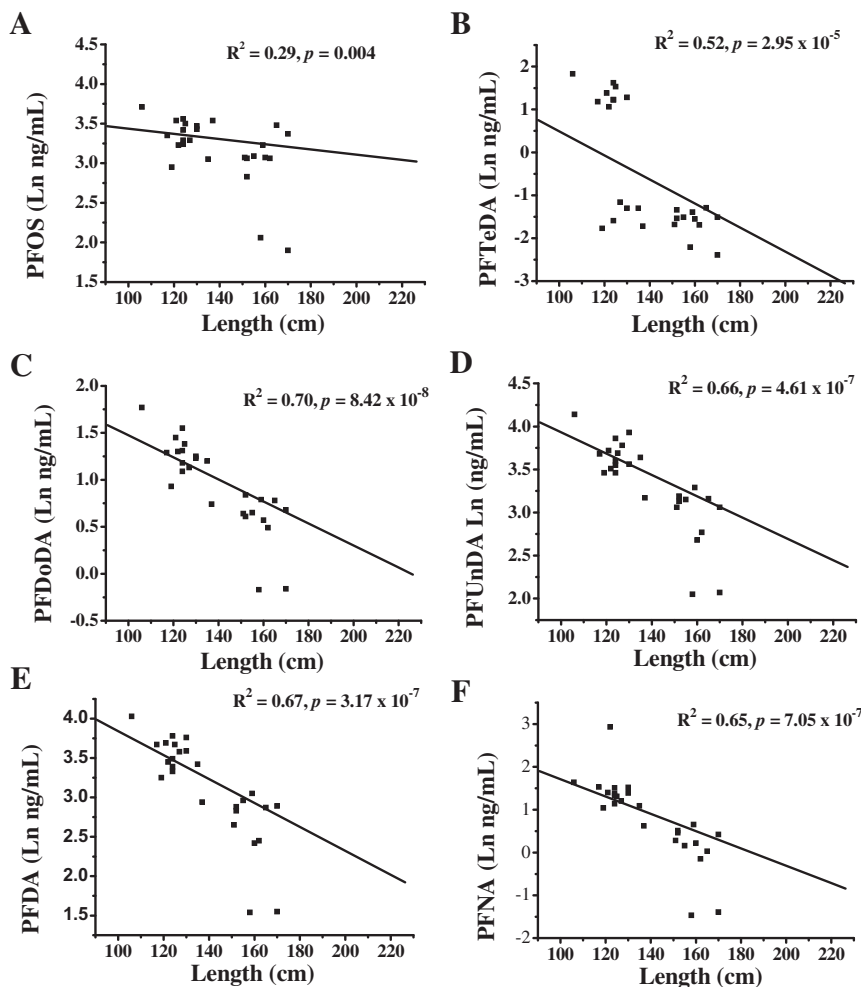

Fig. 2. Linear regression analysis of body length (used as a proxy for age) and PFAS concentrations in female Chinese alligators.
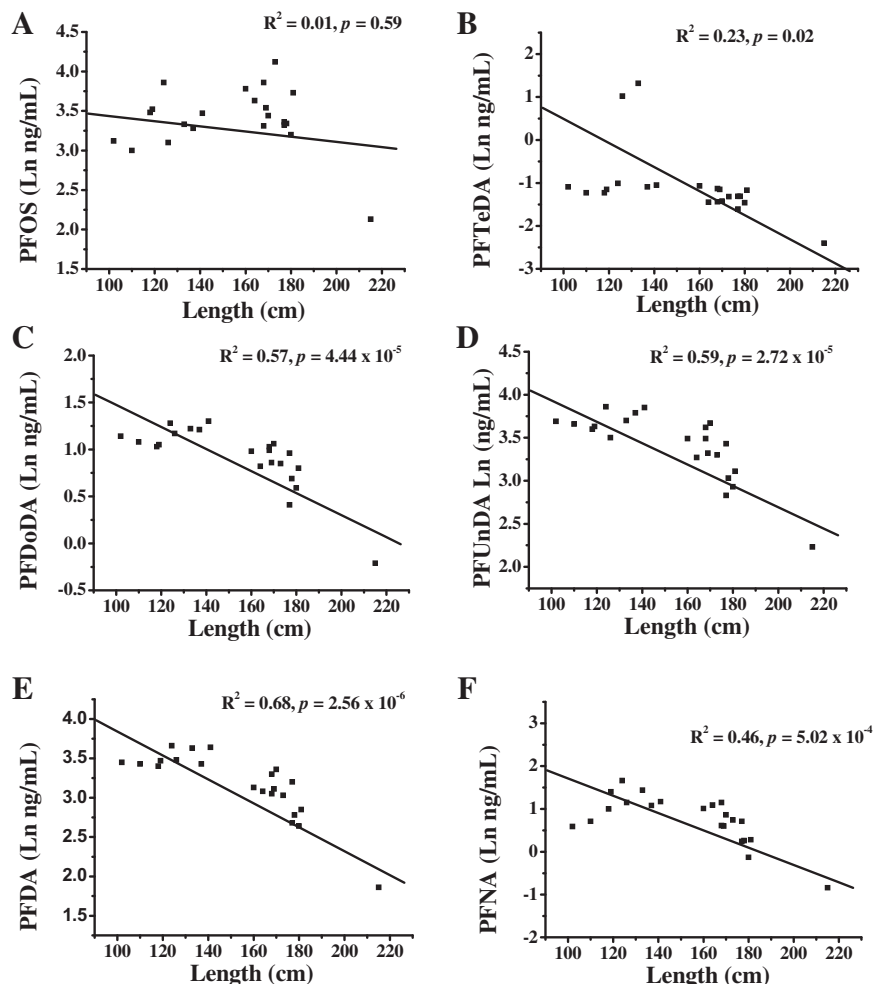
Fig. 3. Linear regression analysis of body length (used as a proxy for age) and PFAS concentrations in male Chinese alligators.

the male subgroup. These results suggest different gender-related bioaccumulation patterns for PFOS in Chinese alligators.

3.4. PFASs in water and fish

PFAS pollution is pervasive in eastern China, including the area surrounding the lower reaches of the Yangtze River (So et al., 2007; Yeung et al., 2008). Chinese alligators reside in environments close to highly industrialized areas and are likely exposed to PFASs via dermal contact, consumption of contaminated water, and ingestion of contaminated food. For reptiles, inhalation of PFASs in air can also be a potential contributor to the observed PFASs exposure burden in alligators. Fourteen PFASs were measured in six kinds of fish samples (whole body homogenates) and water from the pond where the alligators inhabited. Nine PFASs, including PFOS, PFTeDA, PFDoDA, PFUnDA, PFDA, PFNA, PFOA, PFHpA, and PFHxA, were detected in the food samples (SI Table S4). Northern snakehead fish samples had the greatest total PFAS concentrations (36.1 ng/g wet weight (w.w.)), followed by silver carp (16.7 ng/g w.w.) and oriental river prawn (11.2 ng/g w.w.). Different PFASs composition profiles were observed in the fish samples (SI Fig. S3), e.g., in common carp, where the dominant PFAS was PFHxA contributing 38.0% to the total PFASs. Detectable concentrations of PFOS, PFUnDA, PFDA, PFNA, and PFOA were found in pond water (SI Table S4). The dominant PFAS was PFOA, which accounted for 53.4% of total PFASs, followed by PFOS (20.9% of total PFASs) > PFNA (11.5%) > PFDA (8.5%) > PFUnDA (5.8%).

3.5. Bioaccumulation and biomagnifications

The concentrations of PFOS in fish ranged from 0.4 to 7.8 ng/g w.w, which were approximately two to three orders of magnitude greater than concentrations found in surface waters; the BAF for PFOS was in the range of 180–3800 (Table 3). Our values were within the range reported for lake trout exposed to PFOS under laboratory conditions, as well as for wild aquatic organisms in the Great Lakes (Kannan et al., 2005a; Martin et al., 2003a). Similar ranges of BAFs were also obtained for PFUnDA, PFDA, and PFNA. The BAF for PFOA was approximately one or two orders of magnitude lower than that for PFOS (Table 3), and these results were similar to the bioconcentration factor reported for PFOA in rainbow trout exposed under laboratory conditions, which ranged between 4 and 27 (Martin et al., 2003a). The lower BAF of PFOA than that of PFUnDA, PFDA, and PFNA may relate to the low bioaccumulation potential of shorter carbon chain PFCAs than longer ones as well as the shorter half-life of PFOAs in fish.

Since data on the tissue distributions of PFASs were not available, the extrapolation of concentrations detected in serum to the entire body could be not conducted. The BMFs were calculated for the alligator relative to each of its prey items by dividing mean alligator sera concentration with mean concentration of the whole fish or prawn homogenates. Although BMF calculated using whole prey homogenates and alligator serum varied considerably (0.6–200), they were >1 for all analytes in all prey species, except for PFNA in silver carp (BMF Alligator$_{serum}$/Silver carp$_{whole}$ = 0.6)

Table 3
Bioaccumulation factors (BAFs) in fish and biomagnification factors (BMFs) in Chinese alligator food web for PFASs at Anhui Research Center for Chinese alligator Reproduction (ARCCAR).

	PFOS	PFTeDA	PFDoDA	PFUnDA	PFDA	PFNA	PFOA
BAF[a]							
Silver carp/water	3800	n.c.[b]	n.c.	2100	1800	3400	120
Oriental river prawn/water	450	n.c.	n.c.	8500	3000	860	85
Northern snakehead/water	3000	n.c.	n.c.	28,000	9600	2000	42
Common carp/water	180	n.c.	n.c.	610	160	140	n.c.
Tire track eel/water	1100	n.c.	n.c.	2700	820	930	59
Crucian carp/water	240	n.c.	n.c.	1300	450	380	21
BMF[c]							
Alligator/silver carp	3.7	110	6.2	26	17	0.6	n.c.
Alligator/oriental river prawn	31	2.2	2.7	6.5	10	3.0	n.c.
Alligator/northern snakehead	4.6	3.2	1.1	2.0	3.2	1.3	n.c.
Alligator/common carp	76	9.9	17	90	200	18	n.c.
Alligator/tire track eel	13	14	11	20	38	2.7	n.c.
Alligator/crucian carp	58	11	14	44	68	6.7	n.c.

[a] BAFs are based on PFAS measurements in whole fish or prawn homogenates and in water samples.
[b] n.c.: not calculated because concentrations in part of the samples were <LOQ.
[c] BMFs are based on PFAS measurements in whole fish or prawn homogenates and in alligator serum samples.

(Table 3). The BMF calculated by Houde et al. (2006a) using whole prey homogenates and whole body burden dolphin concentration estimation varied between <1 and 30 in the bottlenose dolphin food web at Charleston. Since there is preferential PFAS partitioning between blood and liver, BMFs using sera concentrations for alligators, while using whole body homogenate for prey species, might be overestimated in the present study. The diet species as well as abundance of each species for alligators was not available, so a diet weighted BMF could not be calculated, which affected the evaluation of biomagnifications for alligators.

In conclusion, various levels of PFASs were found in serum of Chinese alligators, demonstrating they were exposed to these chemicals. The PFTeDA, PFDoDA, PFUnDA, PFDA, and PFNA concentrations showed a decreasing trend with the increase in age. However, the present study was limited by a relatively small sample size, which increased the possibility of potential selection biases. Further study with larger sample sizes is necessary to address the influence of age on PFAS accumulation and the biological consequences of these chemicals on this endangered animal.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.04.020.

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