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Bioaccumulation and risk assessment of per- and polyfluoroalkyl substances in wild freshwater fish from rivers in the Pearl River Delta region, South China

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ABSTRACT

Per- and polyfluoroalkyl substances (PFASs) are used in various industries, which results in their ubiquitous occurrence in the environment. This study determined the concentrations of eighteen PFASs in muscle and liver of nine wild freshwater fish species collected from rivers in the Pearl River Delta (PRD) region, South China, and assessed their bioaccumulation and potential health risks to local people. The results showed that eight and twelve PFASs were detected in the fish muscle and liver samples, respectively. Perfluorooctane sulfonate (PFOS) was found to be the predominant PFAS both in muscle and liver with its highest concentrations of 79 ng/g wet weight (ww) in muscle and 1500 ng/g ww in liver, followed by Perfluoroundecanoic acid (PFUnDA) and Perfluorotridecanoic acid (PFTrDA) with trace concentrations. The mean PFOS concentrations in fish muscle and liver tissues of the nine collected species ranged from 0.40 ng/g in mud carp to 25 ng/g in snakehead, and from 5.6 ng/g in mud carp to 1100 ng/g in snakehead, respectively. Significant positive correlations were found among PFASs both in water and fish, indicating a similar pollution source for these PFASs. In tilapia samples, PFOS concentrations showed an increasing trend with increasing length and weight, but no significant difference between genders. Bioaccumulation factors (log BAF) in fish for the PFASs were in the range from 2.1 to 5.0. The calculated hazard ratios (HR) of PFOS for all fishes were in the range of 0.05–2.8, with four out of nine species (tilapia, chub, leather catfish and snakehead) having their HR values more than 1.0. The results suggest that frequent consumption of these four fish species may pose health risks to local population.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs), including perfluorinated carboxylates (PFCAs) and sulfonates (PFSAs), are a class of man-made organic chemicals widely used in industrial applications, such as carpet, metal plating, fire-fighting foams, semiconductor and food packaging, paper and other areas since the mid-20th century (Key et al., 1997; Giesy and Kannan, 2001; Moody and Field, 2000; Lewandowski et al., 2006). After almost a half century use, Giesy and Kannan (2001) first reported the occurrence of PFASs in wildlife, and this raised great concern over scientific community. Subsequently, high bioaccumulation was observed in biota for this group of chemicals; for example, a bioaccumulation factors (BAF) of 23,000 was found for perfluoro-*n*-tridecanoic acid (PFTrDA) in rainbow trout under laboratory exposure conditons (Martin et al., 2003). Meanwhile, adverse effects including hepatotoxicity, developmental toxicity, immunotoxicity and hormonal effects in animals have been proven because of exposure to PFASs (Lau et al., 2007; Peters and Gonzalez, 2011). As of their unique physicochemical properties and persistence, bioaccumulation (biomagnification) and toxic properties (PBT), ever since then, large amount of studies on PFASs especially PFOS and PFOA have been performed worldwide mainly on their occurrence and toxicity. Because of the properties of high solubility of PFASs, most of this group of chemicals would exist mainly in water phase, but some of PFASs could accumulate in fish. It proved ubiquity of this group of chemicals with ng/L levels in surface water (Hansen et al., 2002; Hong et al., 2013), ng/g levels in biota (Giesy and Kannan, 2001; Tao et al., 2006; Bloom et al., 2009), and ng/mL levels in human serum (Hansen et al., 2001). As a result, PFOS and its related chemicals were phased out in the

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United States in 2002, and listed to Annex B of Stockholm Convention which restricted its production and use worldwide UNEP (2009).

Previous studies have reported that air, drinking water, indoor dust and food are the primary pathways for human exposure to PFASs (Fromme et al., 2009;Vestergren and Cousins, 2009; Zhang et al., 2011; Knobeloch et al., 2012). Food consumption is believed to be the major pathway for human exposure to PFASs, contributing more than 60 percent of total lifetime exposure (Tittlemier et al., 2007). In particular, fish has been suggested as the most important source of PFASs exposed to humans through dietary route (Haug et al., 2010).

China is the largest fish production country, with the production volume of 47.5 million tons in 2008, and wild fish (14.8 million tons) accounted for 31.2 percent of the total production (Food and Agriculture Organization of the United Nations, 2010). As a highly urbanized region of the Pearl River Delta (PRD), it would consume more fish than other regions. Kannan et al. (1997) reported that in the PRD, consumption of contaminated fish is one of the major pathways for human exposure to organic pollutants. However, no large-scale study focusing on PFASs in wild freshwater fish samples has been performed in China until now. Moreover, there is scarce information on the risks of PFASs exposure via wild fish consumption in China, especially in the PRD region.

The objectives of this study were: (1) to investigate the contamination levels and profiles of eighteen PFASs (11 PFCAs, 5 PFSAs, 1 perfluoro-1-octansulfonamide (PFOSA) and 1*N*-ethylperfluoro-1-octanesulfonamido acetic acid (*N*-EtFOSAA)) in different fish species collected from rivers of the PRD region; (2) to evaluate the gender-, body weight- and length-related PFASs bioaccumulation in a model fish species (tilapia); and (3) to assess the potential risks of local people exposure to PFASs through fish consumption. The results from this study can help better understand the contamination of PFASs in the rivers of the PRD region and assist local governments to better manage the exposure risks.

2. Materials and methods

2.1. Chemical and reagents

Eighteen PFASs were examined in this investigation, with their full names, abbreviations and formula being given in Table S1. Purities of all the analytical standards were more than 95 percent. PFBA, PFPeA and PFH_XA were purchased from J&K Company (Guangzhou, China), Acros Organics (Geel, Belgium) and Tokyo Chemical Industries (Portland, OR, USA), respectively. PFOA and PFOS were obtained from Accustandard (New Haven, USA). PFHpA, PFNA, PFDA, PFD_oDA and PFTeDA were acquired from Alfa Aesar (Ward Hill, MA, USA), while PFUnDA, PFTrDA, PFBS and PFH_XS were obtained from Sigma-Aldrich (St. Louis, USA). PFHpS, PFDS, N-EtFOSAA and internal standards ($MPFH_XA$ ($^{13}C_2$-PFH_XA), MPFOA ($^{13}C_4$-PFOA), MPFNA ($^{13}C_5$-PFNA), MPFDA ($^{13}C_2$-PFDA), $MPFH_XS$ ($^{18}O_2$-PFH_XS), and MPFOS ($^{13}C_4$-PFOS)) were bought from Wellington laboratories (Guelph, ON, Canada). LC–MS grade ammonium acetate (> 99 percent) was purchased from CNW (Dusseldorf, Germany). Potassium hydroxide was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ammonium hydroxide (10 percent) and acetic acid were bought from Fluka (Germany). HPLC grade methanol (MeOH) was purchased from Merck Corporation (Darmstadt, Germany). The cartridges used for purification were Oasis WAX cartridges (150 mg sorbent, 6 mL size) from Waters (Milford, MA, USA). Ultrapure water was supplied by a Milli-Q system from Millipore (Watford, UK). Individual stock solutions of the target analytes and internal standards were prepared in methanol and stored in polypropylene (PP) bottles at −18 °C.

2.2. Sample collection and sample pretreatment

The study area is shown in Fig. 1, which lists the location of sampling sites in the rivers of the PRD, South China. Fish samples were collected by electroshocking and netting from 11 monitoring sites in the year of 2011–2012. Surface water samples were also collected for two seasons at the same time. Three replicate water samples were collected from each site in each season using a clean stainless steel bucket or polypropylene containers and stored in polypropylene containers with narrow mouths and screw tops. Detailed information about the sampling sites and collected fish species are given in Table 1. The collected fish species in this study included tilapia (*Tilapia aurea*), crucian carp (*Carassius auratus*), common carp (*Cyprinus carpio*), leather catfish (*Clarias fuscus*), snakehead (*Ophicephalus argus*), grass carp (*Ctenopharynodon idellus*), chub (*Hypophthalmichthys molitrix*), mud carp (*Cirrhinus molitorella*), and bream (*Parabramis pekinensis*). All the collected fish samples were kept alive in cold water with oxygen supply and immediately transported to the laboratory after collection. Once arrived in the laboratory, those fish were anaesthetized and skins were removed, and the muscle samples were cut into small pieces. And only muscle and liver samples were used for this study. Each fish sample was individually wrapped in aluminum foil and then put in polyethylene bags. Then the muscle tissues were freeze-dried, ground to fine powder, wrapped in aluminum foil and stored at −18 °C until extraction. Liver samples were wrapped in aluminum foil directly and stored at −18 °C until extraction.

2.3. Sample extraction

The collected water samples were filtered using glass fiber filters (GFF, Whatman, O.D. 47 mm, 0.7 μm), stored in a cold room at 4 °C in darkness and extracted within five days. The water samples (500 mL each) were extracted by solid phase extraction (SPE) using Waters Oasis WAX Cartridges, which is adopted from a previous reported method (Taniyasu et al., 2005), with addition of the internal standards mixture (5 ng each) prior to extraction. Two different extraction methods (alkaline digestion and ion-pairing methods) were used for the extraction of muscle and liver samples in this study, respectively. For the muscle samples, a previous reported alkaline digestion method was used in the extraction (Taniyasu et al., 2005). In brief, 0.2 g of each dried muscle sample (approximately 1.0 g wet sample) was weighed into a 50 mL PP centrifuge tube, followed by addition of 5 ng of each internal standard. Then 10 mL of 10 mM KOH in methanol was added to the tube, which was shaken at 250 rpm for 16 h. After digestion and centrifugation, the supernatant was transferred to a 250 mL PP bottle and diluted to 200 mL with Milli-Q water, which was used for purification with an Oasis WAX cartridge. The cartridge was pre-conditioned with 4 mL 0.1 percent NH_4OH in MeOH, 4 mL MeOH and 4 mL Milli-Q water. After loading, the target compounds were eluted from the cartridge with 4 mL MeOH and 4 mL 0.1 percent NH_4OH in MeOH. Then the eluate was brought to dryness under a gentle stream of nitrogen, and then reconstituted in 500 μL methanol. The final extract was filtered through a 0.22 μm nylon filter into a 1 mL PP snap top vial with a polyethylene (PE) cap and stored in −18 °C until analysis.

For the liver samples, the ion-pairing liquid extraction method was applied in this study for PFASs as described elsewhere (Yeung et al., 2006). Briefly, 0.2 to 0.5 g of each wet liver sample was weighed into a 50 mL PP tube and homogenized by IKA T10 basic ULTRA-TUTTAX homogenizer (Germany) at 30,000 rpm with 2 mL Milli-Q water, 2 mL of 0.25 M sodium carbonate buffer and 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate (TBAHS) solution. After completely homogenized, the PP tube was vigorously shaken for 5 min for extraction. After thorough mixing, 5 mL of methyl-*tert*-butyl ether (MTBE) was added into the tube, and the mixture was shaken again for 20 min. The organic and aqueous layers were separated by centrifugation at 3500*g* for 20 min, and an exact volume of 4 mL of MTBE was transferred into a 10 mL PP tube. Another 5 mL of MTBE was added into the remnant aqueous mixture again, followed by shaking and centrifuging with the above conditions, the supernatant was combined with the first one in the 10 mL PP tube. The MTBE extract was allowed to evaporate to dry under nitrogen and reconstituted in 500 μL of methanol. The final extract was filtered through a 0.22 μm nylon filter into a 1 mL PP snap top vial with a PE cap and stored in −18 °C until analysis.

2.4. Chemical analysis

High performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) was used to determine the concentrations of the target PFASs in the extracts. The instrument used in the analysis was an Agilent 1200 HPLC system interfaced to an Agilent 6460 Triple Quadrupole mass spectrometer that was operated under electrospray negative ionization (ESI-) mode. A 5 μL aliquot of each sample extract was injected into the instrument. The target compounds were separated on a Betasil C18 column (2.1 mm i.d. × 50 mm length, 5 μm; Thermo Hypersil-Keystone, Bellefonte, PA, USA) with a pre-column (2.1 mm, 0.2 μm; Agilent Technologies). The mobile phase used consisted of 2 mM ammonium acetate aqueous solution (solvent A) and methanol (solvent B) at a flow rate of 250 μL/min. The gradient program of the mobile phase was given as follows: 10 percent B at 0 min, increasing linearly to 35 percent B at 0.1 min, 55 percent B at 7 min, and finally to 95 percent B at 17 min and kept for 1 min, then reversing to 10 percent B at 20 min. The capillary voltage was held at 3500 V. Dry and sheath gas flows were maintained at 6 and 12 L/min, respectively. Dry and sheath temperatures were kept at 325 and 350 °C, respectively. The mass spectrometer was operated under multiple reaction monitoring (MRM) mode. The MS/MS mass transition, fragmentor and collision energy of each compound are listed in Table S1.

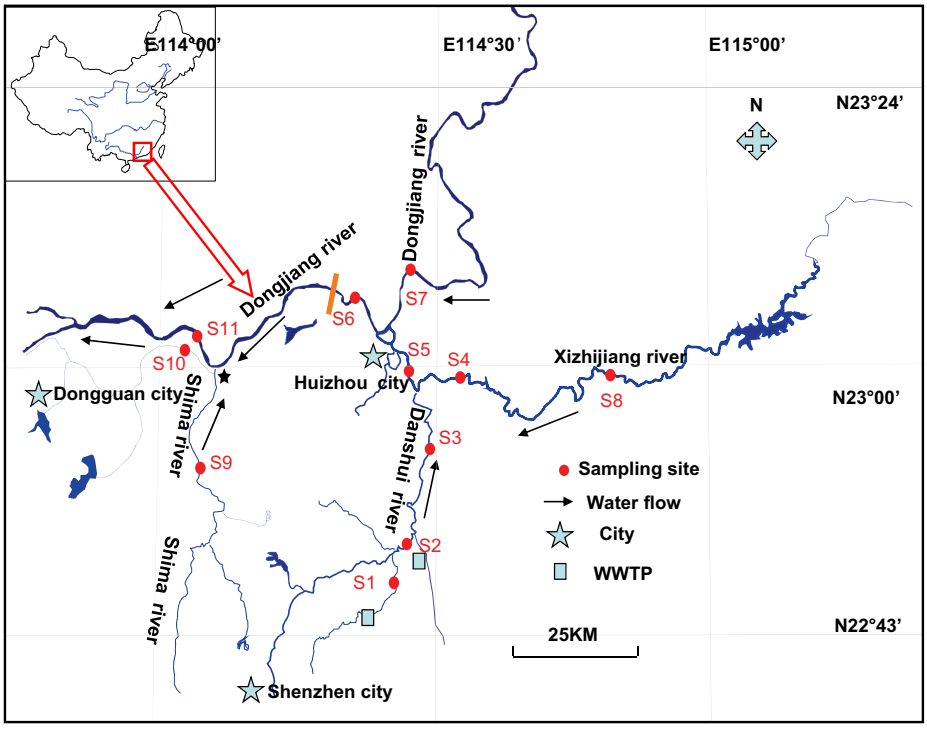

Fig. 1. Location map of the sampling sites in the rivers of the Pearl River Delta (PRD) region, South China.

2.5. Quality control and method performance

Quantitative analysis was performed under MRM mode with the internal standard method Quality assurance/quality control (QA/QC) procedures were followed during the sampling, extraction and analysis. Teflon coated labware and glassware were avoided during the whole process of sampling, pretreatment and analysis to minimize contamination of the samples. PFASs standards, extracts and samples should avoid contacting with any glass containers as these analytes can potentially adsorb to glass surfaces. To reduce instrumental background contamination arising from HPLC or solvents, a ZORBAX SB-Aq trap column (Agilent technologies, 50 × 4.6 mm, 3.5 μm particle size) was inserted in the water-eluent line, immediately above the solvent-mixing cell. Blanks and control samples were run every 7 samples to check for any carryover, background contamination, precision and accuracy of the recovery. The limit of detection (LOD) and limit of quantification (LOQ) of each target compound were defined as 3 and 10 times the signal to noise ratio (S/N), which was calculated by Agilent Masshunter qualitative software. The LOD and LOQ and recoveries of each PFAS in fish tissue and water are given in Table S2.

2.6. Statistical analysis

The concentrations below LOQ were assigned as zero during the calculations. The difference of PFOS concentrations in nine fish species was performed by Kruskal–Wallis H test. A Pearson's correlation analysis was used to examine possible correlations among various PFASs in fish samples. A one-way ANOVA was used to investigate the relationships of PFASs between different fish genders, lengths and weights. All statistical analyses were performed by using the SPSS software (Version 18.0 for windows, SPSS Incorporate, Chicago, IL). Statistical significance was accepted at $p < 0.05$.

3. Results and discussion

3.1. Concentrations of PFASs in water and fish

Surface water and fish samples from 11 sites were analyzed for the PFASs, and the concentrations of the PFASs are presented in Table S3 and Fig. 2, respectively. PFOS was the predominant PFAS compound measured in water with the mean concentrations ranging from 0.17 ng/L at the site S8 to 290 ng/L at the site S1. PFOA was the second predominant PFAS compound with the mean concentrations ranging from 0.21 ng/L at S8 to 22 ng/L at S3. The PFASs with short carbon chains (C4–C9) had much higher detection frequencies and concentrations than those of long carbon chains (C10–C14) (Table S3). Similar contamination patterns for total PFASs in fish were observed for both muscle and liver samples in the eleven sites (Fig. 2). The concentrations for the eighteen PFASs in fish samples at each site are summarized in Tables S4 and S5. For all the muscle samples ($n = 141$) and liver samples ($n = 125$), the eight long chain PFASs ($C \geq 8$) (PFOS, PFNA, PFDA, PFUnDA, PFOSA, PFDoDA, PFTrDA, and PFTeDA) were detected with their detection frequencies mostly exceeding 80 percent, whereas the five short chain PFASs ($C < 8$) (PFBA, PFPeA, PFBS, PFHxA, and PFHxS) were not detected.

PFOS was the predominant compound in both fish muscle and liver, followed by PFUnDA, PFTrDA and PFDA. PFOS contributed 90 percent to the total PFASs in muscle and 92 percent in liver (Fig. S1). The concentrations of PFOS in fish muscle and liver were significantly correlated with its aqueous concentrations (Fig. S2). The highest mean concentration of PFOS in muscle (40 ng/g ww) was observed at S2 (an urban site of Danshui River), which also showed the highest PFOS concentration in surface water. The lowest PFOS mean concentrations (0.26 ng/g ww) in fish muscle was found at S8, which is located in the upstream of the Xizhijiang River. A similar PFOS concentration pattern was also observed for the liver samples, with the highest concentration site at S3 (Fig. 2), which is also located in the Danshui River.

Among the collected nine fish species, the highest PFOS concentrations in muscle and in liver were found in snakehead at 25 ng/g and 1100 ng/g ww. The lowest mean concentrations were found in mud carp at 0.43 ng/g in muscle and 5.6 ng/g in liver, which were nearly 60-fold and 200-fold lower than those in snakehead (Table S4 and S5). Significant differences in PFOS concentrations were observed for most fish species ($p < 0.05$) (Table S4 and S6). In the nine fish species, the mean concentrations (ng/g) of PFOS in muscle and liver had the following increasing trend: mud carp < grass carp < bream < crucian carp < common

Table 1
Basic information of the sampling sites and collected fish.

Sites	Geographic location (N, E)	No. of samples	Species	Length (cm)	Weight (g)
S1	114°26′24″ 22°44′42″	4	Tilapia	11.9–15.3	75.2–150.5
S2	114°27′13″ 22°47′59″	20	Tilapia	11.5–27	70–640
S3	114°29′28″ 22°56′38″	19	Tilapia	16–27	150–580
		4	Snakehead	26–28	192–268.8
		3	Leather catfish	31.6–34.8	249.9–350.8
S4	114°64′68″ 22°97′73″	7	Grass carp	19–33	100–625
		5	Crucian carp	12–20.6	90–270
		2	Common carp	21–30.3	270–1020
S5	114°27′48″ 23°03′10″	4	Tilapia	14–21	80–260
		5	Chub	26–40	270–1240
		2	Crucian carp	19.6–23.5	300–400
		1	Common carp	36	1350
S6	114°21′42″ 23°08′53″	3	Tilapia	14.4–18.7	120–250
		2	Chub	33–38	660–740
		7	Crucian carp	12.6–21.5	90–410
		2	Common carp	35–39.5	1350–2700
S7	114°27′19″ 23°10′51″	9	Tilapia	11.5–22	125–280
		3	Grass carp	25.4–27.0	320–340
		1	Chub	31	580
		4	Crucian carp	17.5–21.1	230–290
S8	114°94′39″ 23°03′41″	3	Tilapia	13–20	90–300
		6	Common carp	23.4–30	380–620
		3	Mud carp	21.7–27.7	175–516
S9	114°07′34″ 22°91′28″	3	Tilapia	12.5–14.7	80–145
S10	114°08′26″ 23°06′19″	12	Tilapia	11.3–21.5	65–300
S11	114°09′16″ 23°07′72″	1	Tilapia	23	490
		1	Chub	41.5	1550
		1	Common carp	34	720
		4	Bream	24.5–30	300–550

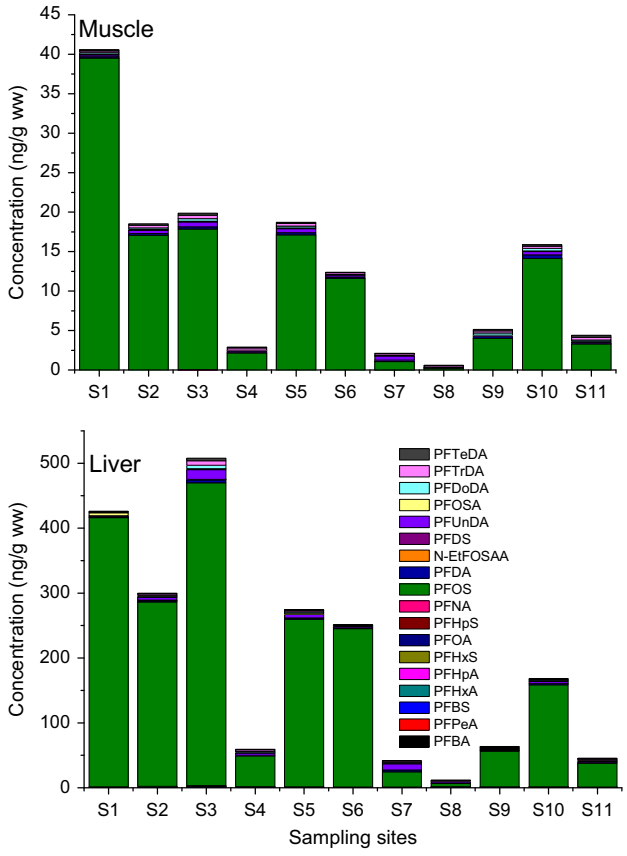

Fig. 2. The PFASs concentrations in muscle and liver of fish from the sampling sites of the PRD rivers.

carp < tilapia < chub < leather catfish < snakehead in muscle, and mud carp < bream < crucian carp < grass carp < common carp < chub < tilapia < leather catfish < snakehead in liver.

PFUnDA was the second dominant PFAS, with its concentrations ranging from < 0.03 to 2.4 ng/g and a mean concentration of 0.38 ng/g in muscle, and from < 0.12 to 57 ng/g with a mean concentration of 6.3 ng/g in liver. PFUnDA contributed 3 percent to the total PFASs in both muscle and liver. PFTrDA is the third largest contributor in both muscle and liver, with concentrations ranging from < 0.03–1.1 ng/g to 0.27–22 ng/g, and contributed only 2 percent and 1 percent to the total PFASs, respectively (Table S4). The relative higher concentrations of these two long chain PFCAs in fish could be due to their relatively higher bioaccumultive ability, higher concentrations in water and site-specific or fish species-specific bioaccumulation properties (Table S3).

Some previous studies have also reported the occurrence of PFOS in fish globally with the concentrations ranging from a few ng/g to thousands ng/g level (Senthilkumar et al., 2007; Delinsky et al., 2010; Berger et al., 2009; Quinete et al., 2009; Becker et al., 2010; Schuetze et al., 2010; Labadie and Chevreuil, 2011; Malinsky et al., 2011; Murakami et al., 2011; Zhang et al., 2011; Shi et al., 2012; Hloušková et al., 2013), and a comparison of PFOS concentrations in fish is presented in Table S7. In general, the concentrations of PFOS in fish muscle of this region (< 0.03–79 ng/g) are higher than those reported in fish from most Asian countries, such as Vietnam, Malaysia and Beijing of north China, where its concentrations ranged from 0.20 to 2.3 ng/g, and were almost at the same level with those in most European countries, such as Sweden, Czech and Germany (0.97–23 ng/g). But the PFOS concentrations from the present study were much lower than the fish from the Mississippi River (28.5–382 ng/g) and Minnesota Rivers near 3 M Company (Former biggest fluorochemical plant), where the maximum concentration in muscle was as high as 2000 ng/g. In fish liver, the PFOS concentrations (0.95–1500 ng/g) in the present study were higher than in those in Japan, Vietnam Malaysia and Germany with the concentrations ranging from 2.35 to 123 ng/g ww (Becker et al., 2010; Murakami et al., 2011). The varied concentrations of PFASs measured in fish species at different sites in the present study can be explained by various factors such as the different PFASs concentrations in water,

different chemical properties, species-specific bioaccumulation characteristics and different dietary habitat.

3.2. Tissue distribution

The present study clearly showed higher PFASs concentrations in liver than in muscle of fish (Fig. 2). This is in good agreement with previous studies performed on various fish species such as rainbow trout, grass carp, common carp, snakehead, and tilapia (Martin et al., 2003; Becker et al., 2010; Shi et al., 2012). This phenomenon might be explained by the high binding affinity of PFASs for liver fatty acid-binding protein (Luebker et al., 2002).

The ratios of PFASs in liver to muscle of fish can be calculated to assess the accumulation of PFASs. The liver/muscle concentration ratios for PFOS were found to range from 6.9 in crucian carp to 42 in snakehead, which are lower than the value of 61.5 in Chinese sturgeon (Peng et al., 2010) and basically at a similar level to the value of 10 in fish from Mediterranean Sea (Nania et al., 2009) and 9.5 for chub from Roter Main River in Bayreuth, Germany (Becker et al., 2010). The mean liver/muscle ratios for PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA in the present study were 3.0–23, 5.0–30, 3.7–32, 2.8–25 and 2.0–34, respectively. These values are found lower than those for PFOS, which is consistent with a previous report by Shi et al. (2012), indicating that PFOS has a stronger accumulation potential in liver when compared with the PFCAs. A significant positive correlation was found for PFOS concentrations between liver and muscle of all fish ($r=0.759$, $p<0.001$), so did the other detected PFASs. The liver/muscle concentration ratios of PFOS followed the order: crucian carp $<\cap$ bream $<$ mud carp $<$ chub $<$ tilapia $<$ common carp $<$ leather catfish $<$ grass carp $<$ snakehead.

Pearson's correlation analysis showed significant correlations among most PFASs found both in muscle and liver (Table S8). For example, PFOS was positively correlated with some other compounds (PFHpS, PFDA, PFDS, PFDoDA, PFUnDA, PFTrDA and PFTeDA), thus PFOS could be used as an indicator compound for other PFASs. This relationship was also found in previous studies (Yeung et al., 2006; Powley et al., 2008;). This may also indicate similar pollution sources for these compounds (So et al., 2007).

3.3. Gender-, length- and weight-related PFASs bioaccumulation in tilapia

Tilapia was the most abundant fish investigated in this study ($n=78$). Thus a detailed analysis was performed for possible gender-, length- and weight-related accumulation of PFASs in tilapia. The PFASs concentrations in muscle were grouped by gender (31 female and 31 male), length (<15 cm, 15–20 cm, >20 cm), and weight (<150 g, 150–260 g, >260 g). Since some tilapia's gender was not identified, the total gender samples were less than the total number of tilapia. One-way ANOVA analysis was applied for gender, length and weight-related PFASs bioaccumulation data. The relationships of individual PFAS concentrations in tilapia by gender, length and weight are shown in Fig. 3.

In muscle, male tilapia had significantly higher PFTeDA concentrations than females ($p=0.015$), but no significant differences were observed for other PFASs *P*. In liver, PFNA and PFDS had significantly higher concentrations in males than females, and no significant differences were observed for the other PFASs. This is consistent with some previous studies on PFOS concentrations between genders with no significant correlations being identified between males and females of harbor seals and harbor porpoises, respectively (Kannan et al., 2002; Keller et al., 2005; Van de Vijver et al., 2007; Ahrens et al., 2009). However, Kannan et al. (2005) found that PFOS concentrations in male snapping turtles were higher than females, but Van de Vijver et al. (2003) found higher PFOS concentrations in female harbor porpoises.

As shown in Fig. 3, the concentrations of PFOS, PFUnDA and PFOSA in muscle gradually increased with length ($p<0.05$). In liver, the concentrations of PFOS, PFOSA and PFTeDA gradually increased with length ($p<0.05$).

The weight related PFASs bioaccumulation patterns were found for two PFASs in muscle and 5 PFASs liver. The concentrations of PFOS and PFUnDA in muscle significantly increased with the weight ($p<0.05$), and PFOS, PFUnDA, PFDoDA, PFTrDA and PFTeDA in liver significantly increased with weight ($p<0.05$). PFOS concentrations increased dramatically from <150 g group to 150–260 g group (Fig. 3). The length- and weight-related PFASs bioaccumulation in fish could be due to ingestion of more food by the larger body fish or different diets for larger individuals.

3.4. Bioaccumulation factors (BAFs) of PFASs

Bioaccumulation factors were calculated based on the concentrations of PFASs in tissue and water (Table S9). According to the current data, the BAF values were calculated for individual PFASs if available. Since only PFASAs with more than nine perfluoroalky carbons, and only one sulfonate PFOS were detected in muscle, the BAFs for the other short chain PFASs were not calculated and these compounds are considered to have no bioaccumulation ability. Log BAF_{muscle} for the collected fish ranged from 1.8 of PFNA (snakehead) to 3.5 of PFUnDA (snakehead). The mean log BAF_{muscle} of PFOS in the nine fish species was in the range of 2.7–3.4, whereas the log BAF of PFOA with eight carbons was not calculated as it was not detected in the muscle samples. For all fish species, the log BAF increased with increasing carbon-chain length (Fig. 4).

For longer-chain PFASAs, their log BAF_{liver} values were equal to or greater than that of PFOS (Fig. 4). This is consistent with the trend observed in wild aquatic organisms in the Great Lakes and chub from Orge River (Kannan et al., 2005; Labadie and Chevreuil, 2011). The log BAF_{muscle} values for PFOS (2.7–3.4) were lower than those observed for European chub (3.7) (Becker et al., 2010), European eel (3.5) (Kwadijk et al., 2010) and Lake trout (3.8–4.4) (Furdui et al., 2007). This could be explained by site or species-specific behaviors or different dietary habits.

In the liver samples, only those PFASAs with more than eight perfluoroalkyl carbons, and PFSAs with more than seven perfluoroalky carbons were detected. Log BAF_{liver} ranged from 2.2 for PFOA (leather fish) to 5.0 for PFUnDA (snakehead). The mean log BAF_{liver} of PFOS in nine fish species was in the range of 3.5–4.6, which are approximately one log unit higher than in muscle. The log BAF_{liver} of PFOS is at the same level as that found by Labadie and Chevreuil. (2011) for European chub with log BAF_{liver} of 4.3 and slightly higher than that for rainbow trout (log $BAF_{liver}=3.7$) (Martin et al., 2003).

The positive correlations between the log BAF values of PFASs and the length of the perfluoroalkyl chain ($p<0.05$, $r^2>0.93$) found in the present study (Fig. 4) are consistent with some previous studies (Martin et al., 2003; Hart et al., 2008; Kelly et al., 2009; Kwadijk et al., 2010; Labadie and Chevreuil, 2011). PFASs with longer carbon chain exhibit higher protein water partition coefficients (K_{pw}), thus resulting in higher bioaccumulative ability for the long-chain PFASs (Kelly et al., 2009).

3.5. Risk assessment through fish consumption

As demonstrated previously, food consumption, especially fish consumption is a major pathway for human exposure to PFASs (Falandysz et al., 2006; Ericson et al., 2008; Fromme et al., 2009; Berger et al., 2009; Haug et al., 2010; Schuetze et al., 2010; Zhang et al., 2011). To assess the potential health risks to human,

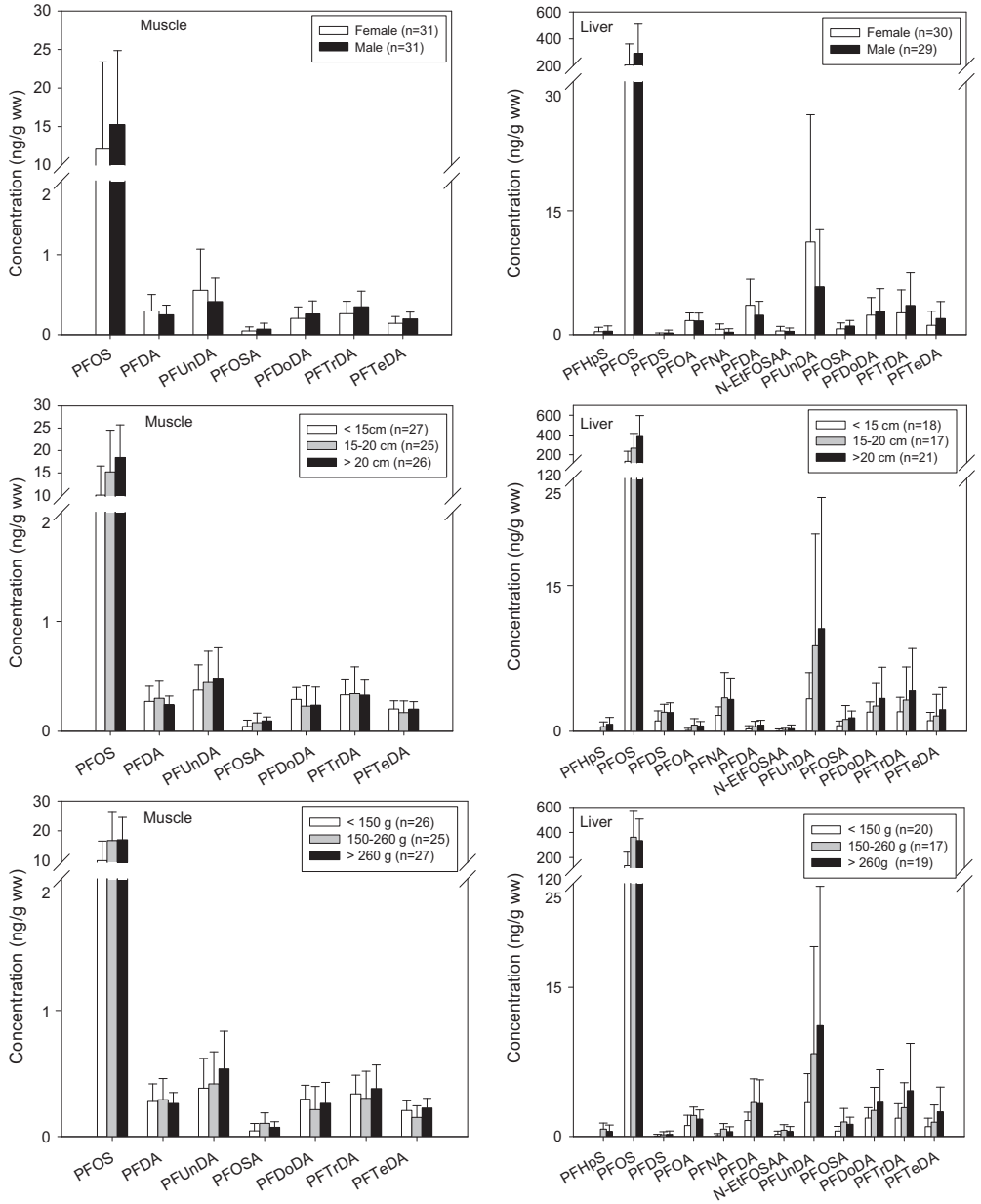

Fig. 3. The PFASs concentrations in tilapia with different genders, lengths and weights (mean ± standard deviation).

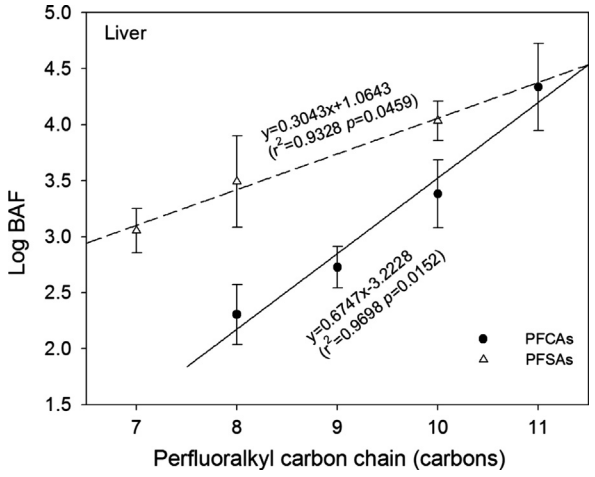

Fig. 4. Correlation between the log BAF and the perfluoroalkyl chain length of PFASs in liver of tilapia. The error bars represent the standard deviations.

exposure concentrations were compared to benchmark dose of a chemical via fish consumption. Hazard ratio (HR) was calculated by dividing the average daily intake (ADI) against Reference Dose (RfD). A HR value greater than 1 suggests that the average exposure level exceeds the benchmark concentration. Average daily intake (ADI) of the chemical was calculated based on the following equation:

ADI = PFAS concentration × fish consumption [g/kg body weight/d]

Assuming an average human body weight of 60 kg and muscle being the only consumed tissue, the amount of fish consumption is about 60 kg per person per year in Hong Kong (Dickman and Leung, 1998). Taking geographical location and eating habits of life into account, the same fish consumption rate was used for the calculation in this region. Since PFOS was the predominant PFAS in the fish collected from the PRD region, risk assessment was only performed for this compound. The RfD for PFOS is 0.025 μg/g/d, which was established on the basis of the rat chronic carcinogenicity studies (Thayer, 2002). The ADI of PFOS of all nine fish species

Table 2
Average daily intake (ADI) and hazard ratio value (HR) for local residents with PFOS exposure via fish consumption.

Fish name	Concentration (ng/g ww)	ADI (ng/kd/d)	HR
Bream	3.6	9.8	0.39
Crucian carp	5.3	15	0.58
Common carp	8.7	24	0.95
Chub	15	40	1.6
Mud carp	0.43	1.2	0.05
Tilapia	14	38	1.5
Snakehead	25	69	2.8
Leather catfish	17	47	1.9
Grass carp	1.7	4.7	0.19

Remark: HR was calculated based on PFOS concentrations in muscle by assuming that muscle is the only consumption tissue.

was calculated to be 31 ng/kg/d for the PRD people. This value is much greater than those reported in Sweden (0.62 ng/kg/d) (Berger et al., 2009), Guangzhou (2.8 ng/kg/d), Zhoushan (1.7 ng/kg/d) (Gulkowska et al., 2006), Hong Kong (2.4 ± 2.9 ng/kg/d), Xiamen (5.1 ± 4.7 ng/kg/d) (Zhao et al., 2011) and Norway (0.78 ng/kg/d) (Haug et al., 2010). This reflects the combination of both high (but not exceptionally high) PFOS levels and relatively high fish consumption rates in this region.

The ADI and HR values of the nine fish species are summarized in Table 2. The HR values for PFOS in chub, tilapia, snakehead and leather catfish were 1.6, 1.5, 2.8 and 1.9, respectively. This indicates that consumption of the four fish species in the region could pose high risks to the local population. However, the HR values for the other five fish species were all less than 1, ranging from 0.05 to 0.95, indicating minimal risks to the local population.

4. Conclusion

The present study showed detection of eighteen PFASs at various concentrations in nine fish species collected from the rivers in the PRD region. The PFOS, PFOSA and PFUnDA concentrations in fish showed an increasing trend with increasing fish length and weight. PFOS was the predominant PFAS compound found both in muscle and liver, followed by PFUnDA and PFTrDA. The PFOS concentrations in fish were strongly correlated to the concentrations in water. Among the nine species, snakehead showed the highest PFOS concentrations, followed by leather catfish, with mud carp having the lowest concentrations. PFASs are more bioaccumulative in liver than in muscle for all the studied fish. The BAF values for PFASs were positively correlated with the perfluoroalkyl carbon numbers. Meanwhile, based on hazard ratios for PFOS, frequent consumption of some contaminated fish such as wild chub, tilapia, snakehead and leather catfish collected from this region could pose potential health risks to local population.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2014.05.031.

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