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Perfluoroalkyl substances (PFASs) in edible fish species from Charleston Harbor and tributaries, South Carolina, United States: Exposure and risk assessment

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ABSTRACT

Concentrations of 11 PFASs were determined in muscle and whole fish for six species collected from Charleston, South Carolina (SC) for the assessment of potential health risks to humans and wildlife. Across all species and capture locations, total PFAS levels in whole fish were significantly higher than fillets by a factor of two- to three-fold. Mean ΣPFAS concentrations varied from 12.7 to 33.0 ng/g wet weight (ww) in whole fish and 6.2–12.7 ng/g ww in fillets. For individual whole fish, ΣPFASs ranged from 12.7 ng/g ww in striped mullet to 85.4 ng/g ww in spotted seatrout, and in fillets individual values ranged from 6.2 ng/g ww in striped mullet to 27.9 ng/g ww in spot. The most abundant compound in each species was perfluorooctane sulfonate (PFOS), comprising 25.5–69.6% of the ΣPFASs. Striped mullet had significantly lower relative amounts of PFOS compared to all other species and higher relative amounts of PFUnDA compared to Atlantic croaker, spotted seatrout, and spot. Unlike whole fish, PFAS levels in fillets varied significantly by location with higher ΣPFOS from the Ashley River than the Cooper River and Charleston Harbor, which reflects the levels of PFASs contamination in these systems. In whole fish, differences in relative concentrations of PFOS, PFNA, and PFDA occurred by capture location, suggestive of different sources. PFOS concentrations for southern flounder and spotted seatrout fillets were within the advisory range to limit fish consumption to 4 meals a month. PFOS levels exceeded screening values to protect mammals in 83% of whole fish examined and represent a potential risk to wildlife predators such as dolphins.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are a family of fluorine-containing synthetic compounds widely used in consumer and industrial applications (Buck et al., 2011). Because their chemical structure produces a unique ability to repel both oil and water these chemicals have many applications including coatings for paper, packaging, surface protection used on carpet and clothing to resist stains and water, nonstick coatings on cookware, industrial surfactants, and manufacture of fire-resistant foams (Lehmler, 2005). Despite being in use for over 50 years, they were only first detected in wildlife in 2001 (Giesy and Kannan, 2001) and are highly persistent in the environment and bioaccumulate in wildlife (Houde et al., 2011). The most commonly detected PFASs are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). Human biomonitoring studies found PFOS and PFOA in the blood of the general human population, indicating that their exposure is widespread (Kannan et al., 2004; ATSDR, 2018).

The primary non-occupational route of PFAS exposure is diet, and fish and other seafood contain the highest concentrations (Zhang et al., 2011; Domingo and Nadal, 2017). Numerous studies have confirmed that fish is an important dietary source of PFASs in many areas of the world (Falandysz et al., 2006; Haug et al., 2010; Rylander et al., 2009; Exposure Science in the 21st Century, 2012; Yamaguchi et al., 2013). These concerns are amplified in certain populations such as the Greenlandic Inuits whose traditional diets consist of fish and marine mammals (Lindh et al., 2012). Olsen's (2009) review of biomonitoring in higher exposed populations focused on occupational exposures from

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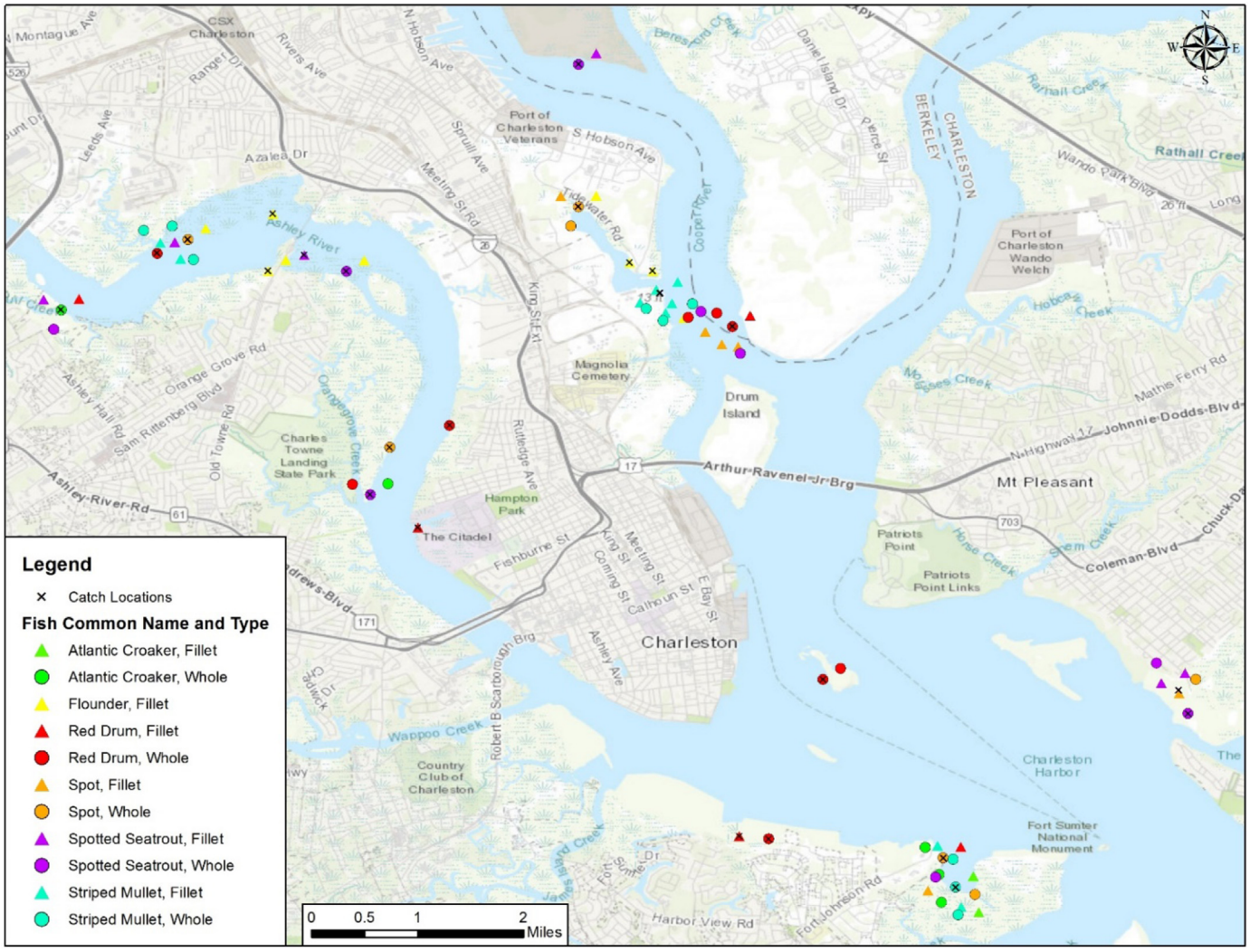

Fig. 1. Capture location for all study samples by type (whole or fillet) and by species (Atlantic croaker, red drum, spot, spotted seatrout, striped mullet, southern flounder). The study included 17 individual capture site (6 Charleston Harbor, 7 Ashley River, and 4 Cooper River sites) and these sites included multiple fish captures. The capture location for fish caught at the same site was perturbed to allow all fish to be visible on the map and the actual geographic location for each capture site is marked by a black X.

production and use of PFAS and also in fishermen since fish is considered an important source of elevated PFOS serum concentrations. Hölzer et al. (2011) established a dose-response relationship between fish consumption and PFOS body burden finding serum PFOS concentrations were two to three-fold higher among individuals consuming at least three fish per month than those who did not consume fish. Further, commercial fisherman on Tangxun Lake in China had the highest serum PFOS levels in the upper ranges of occupationally exposed workers (Zhou et al., 2014). Recently, Christensen et al. (2017) investigated associations between specific seafood consumption and PFAS levels among the U.S. general population. They found that even though overall fish consumption levels were low among National Health and Nutrition Examination Survey (NHANES) participants, fish and shellfish intake were both associated with elevated levels of multiple PFASs, with differences by specific type of fish or shellfish. While certain PFASs in the U.S. population are declining over time, possibly reflecting limitation or elimination of certain exposure sources, levels of other PFASs are steady or increasing over time (CDC, 2018; Bloom et al., 2009). Thus, fish remains an important ongoing source of exposure and warrants examination.

Fish are an increasingly important part of the human diet and fish consumption has increased by about 30% in the United States over the last several decades (Loke et al., 2012). While certain PFASs (e.g., PFOS, PFOA) have been discontinued in the U.S., they are still produced in other parts of the world and imported into the U.S. Concern exists on the manufacturing of shorter-chain replacements and PFAS precursors produced in the U.S. that can break down to persistent degradation products. A gap exists in our current knowledge of how these emerging contaminants affect not only marine mammals, but also the health of humans, especially those who have increased exposure from consumption of local seafood in coastal areas.

PFASs have become a class of chemicals of considerable concern to human and animal health. Evidence from human epidemiological studies demonstrate associations between PFOA exposure and high cholesterol, adverse reproductive and developmental effects, altered liver enzymes, thyroid disorders, immune alterations and pregnancy hypertension (Lau et al., 2007; DeWitt et al., 2012). PFOA has also been found as a possible carcinogen to humans by the World Health Organization's International Agency for Research on Cancer (IARC, 2016). A review by Olsen et al. (2009) indicates PFOS causes developmental and reproductive effects, thus special concerns exist for vulnerable populations (i.e., fetus, infants, children). Toxicological studies in laboratory animals also produced reproductive, developmental and systemic effects (Seacat et al., 2003; DeWitt, 2015).

Some of the highest PFASs found globally in marine mammals have been observed in bottlenose dolphins from Charleston with levels comparable to those of occupationally exposed humans (Houde et al., 2005; Fair et al., 2010, 2012). The bottlenose dolphin is a long-lived top-level predator with high site fidelity to estuarine areas, hence it is a useful sentinel species for monitoring the health of the environment and signaling emerging public health issues (Bossart, 2011). Based on the high PFAS levels reported in Charleston dolphins, local sediment samples were analyzed and found to be higher than any other urban U.S. area with over half of the sites exceeding the median global PFOS sediment concentration (White et al., 2015). Despite the high levels of PFASs found in Charleston dolphins, little information is available on PFAS contamination in fish from this area. The African American and Gullah/Geechee population of fishers in the Charleston estuarine area share similarities with the dolphin in commonly consumed fish species (Pate and McFee, 2012; Ellis, 2013). Since the Gullah/Geechee African American population participate in local subsistence fishing they may be a potentially vulnerable and susceptible group to pollutant exposure from consumption of seafood. Therefore, it is important to determine the levels of PFASs in fish species from this region. Fish contaminant data by species for specific rivers and tributaries are necessary to provide risk analysis for local populations of recreational anglers. Our companion paper investigated liphophilic persistent organic pollutants (POPs) (i.e., polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), pesticides) that are found high in fish from Charleston, SC (Fair et al., 2018). Fewer studies have investigated PFASs in fish compared to POPs, and PFASs differ from POPs as they are not lipophilic but have protein affinity (Butt et al., 2010).

The objectives of this study were: 1) to investigate the contamination levels and profiles of 11 PFASs in fish species that dolphins and humans consume in the Charleston SC area, and 2) to estimate dietary exposure to PFASs by fish consumption and assess risks in humans and dolphins. The results of this study provide important information about contamination of PFASs in fish from the rivers and harbor of Charleston and assist local governments to manage exposure risks of wildlife and humans.

2. Methods

2.1. Sample collection and preparation

The study area is shown in Fig. 1, which lists the location of the sampling sites in Charleston Harbor (CH), the Ashley River (AR) and Cooper River (CR). Fish were collected by the South Carolina Department of Natural Resources as part of their trammel net Inshore Fisheries monitoring program of estuarine fish (Arnott et al., 2010) or directed sampling. During 2014 a total of 39 fish from the 3 sites (CH = 15; AR = 13; CR = 11) were collected for contaminant analysis of whole fish and 37 fish (CH = 10; AR = 10; CR = 17) were collected for fillet contaminant analysis (Table 1). Five known dolphin prey fish species (Pate and McFee, 2012) were collected: Atlantic croaker (*Micropogonias undulatus*); red drum (*Sciaenops ocellatus*); spot (*Leiostomus xanthurus*), spotted seatrout (*Cynoscion nebulosus*) and striped mullet (*Mugil cephalus*). These same 5 fish are also commonly caught and consumed by the Gullah-Geechee African American community and other recreational anglers. We also sampled one additional fish species, southern flounder (*Paralichthys lethostigma*), based on fish consumption questionnaire data from the Gullah-Geechee community (Ellis, 2013). Total length and weight of each fish and geographic coordinates were collected (Supplemental Table 1). Each fish specimen was wrapped individually in heavy aluminum foil, placed in a polyethylene bag, sealed, held on ice for 3–6 h and then frozen at −20 °C until sample preparation and analysis. PFAS analyses were performed on tissue homogenate prepared from individual whole fish representing fish consumed by dolphins. For assessment of human consumption, individual skin-on fillets were analyzed. It is common methodology that fillets with skin are used for contaminant monitoring studies. Also, skin is left on and eaten by local subsistence fishers. A fillet included the flesh tissue and skin from head to tail beginning at the mid-dorsal line including the belly flap. Filleting was conducted on cutting boards covered with heavy duty aluminum foil and changed between samples. Whole fish and fillet samples were ground using a Hobart Grinder (Elk Grove Village, IL). All equipment was thoroughly cleaned with detergent, rinsed in isopropanol and washed with distilled water before each specimen was processed.

Table 1
Fish demographics across all sample and by whole fish or fillet. Categorical variables are reported as n (%) and continuous variables are reported as mean (SD).

	All Sample (n = 76)	Whole Fish (n = 39)	Fillets (n = 37)
Species			
Croaker	7 (9.22)	5 (12.8)	2 (5.41)
Flounder	9 (11.8)	–	9 (24.3)
Mullet	18 (23.7)	9 (23.1)	9 (24.3)
Red Drum	14 (18.4)	9 (23.1)	5 (13.5)
Spot	13 (17.1)	7 (18.0)	6 (16.2)
Seatrout	15 (19.7)	9 (23.1)	6 (16.2)
Location			
Charleston Harbor	25 (30.9)	15 (38.5)	10 (27.0)
Ashley River	23 (28.4)	13 (33.3)	10 (27.0)
Cooper River	28 (36.8)	11 (28.2)	17 (46.0)
Length (mm)			
Croaker	207.6 (55.7)	206.2 (39.0)	211.0 (5.66)
Flounder	392.6 (47.3)	–	392.6 (47.3)
Mullet	259.7 (35.2)	235.4 (31.7)	283.0 (47.5)
Red Drum	441.1 (94.6)	464.3 (74.6)	399.2 (120.6)
Spot	231.7 (93.5)	177.9 (39.8)	294.5 (101.4)
Seatrout	390.3 (62.4)	412.0 (66.8)	357.7 (40.5)
Weight (g)			
Croaker	129.4 (32.0)	136.2 (30.1)	112.5 (0.71)
Flounder	757.6 (304.7)	–	757.6 (304.7)
Mullet	246.3 (48.1)	267.1 (41.1)	225.6 (47.5)
Red Drum	1092.4 (735.6)	1217.3 (212.0)	867.4 (795.0)
Spot	242.2 (252.8)	122.1 (58.7)	382.2 (325.0)
Seatrout	527.3 (153.3)	577.1 (134.7)	452.5 (159.8)

2.2. Analysis of PFASs in fish samples

The following 11 PFASs were measured: PFCAs (perfluorinated carboxylic acids) included PFDA (perfluorodecanoic acid); PFUnDA (perfluoroundecanoic acid); (PFDoDA perfluorododecanoic acid); PFHpA (perfluoroheptanoic acid); PFHxA (perfluorohexanoic acid); PFNA (perfluorononanoic acid); PFOA (perfluorooctanoic acid); PFPeA (perfluoropentanoic acid); and PFSAs (perfluorinated sulfonates) included: PFHxS(perfluorohexanesulfonate); PFOS (perfluorooctane sulfonate); PFOSA (perfluorooctanesulfonamide). Fish samples (0.9–1.2 g ea.) were homogenized with 5 mL of ultrapure Milli-Q water. One mL of the homogenized sample was transferred into a 15 mL polypropylene tube (PP tube) and spiked with 50 μL of 5 ng internal standard (IS) mixture ($^{13}C_4$-PFOSA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, $^{13}C_3$-PFPeA, $^{13}C_2$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_4$-PFOA, $^{13}C_2$-PFDA, $^{13}C_2$-PFNA, $^{13}C_2$-PFUnDA, and $^{13}C_2$-PFDoDA). To this mixture, 0.25 M sodium carbonate buffer (2 mL) and 0.5 M tetrabutylammonium hydrogen sulfate (TBAHS) solution (1 mL, adjusted to pH 10) were added and mixed thoroughly. The extraction of target compounds was performed by the addition of 5 mL of methyl-tert-butyl ether (MTBE) with vigorous shaking for 40 min using a mechanical shaker. The sample was centrifuged at 4000 rpm for 5 min, and the ether layer was separated and transferred into a new PP tube. The extract was evaporated to near-dryness under a gentle stream of nitrogen and the residue was reconstituted with methanol (1 mL), vortexed for 30 s and transferred into an autosampler vial. The PFASs analysis was carried out using a LC-MS/MS (Agilent LC 1100 coupled with AB SCIEX API-2000 triple quadrupole mass spectrometry) under

negative electrospray ionization conditions (Software: Analyst). The optimized mass spectrometric and chromatographic parameters were reported elsewhere (White et al., 2015).

2.2.1. Quality assurance/quality control (QA/QC)

A 10-point calibration curve with concentrations that ranged from 0.05 to 50 ng/g was constructed for all target chemicals and the linear regression coefficients (R) were > 0.987. A pure solvent (methanol) and a midpoint calibration standard (1 ng/g) were injected between every 10 samples to verify carryover (if any) and instrumental drift in sensitivity. An isotope-dilution method was used for the quantification of PFASs in fish. Process blanks, blank spikes, matrix spikes and sample duplicate analysis were performed for each batch (n = 20). The limits of quantification (LOQ; S/N ≥ 10) were ranged between 0.06 and 0.88 ng/g ww. There were no measurable levels of (> LOQ) target compounds present in the process blanks. The spike recoveries (20 ng) from the water blank and matrix (fish tissue) ranged between 84% and 124% and 90–118%, respectively, for all target compounds. Duplicate analysis of randomly selected samples yielded a coefficient of variation of 3–15% for the measured concentrations of PFASs.

2.3. Statistical analysis

Descriptive statistics were calculated for whole fish and fillet samples for biological variables (weight and length) and for total PFASs; perfluorinated sulfonates (PFSA) which included PFOS, PFOSA, and PFHxS; perfluorinated carboxylic acids (PFCA) comprised of PFOA, PFNA, PFDA, PFUA, PFDoA, PFHxA, PFHpA, and PFUnDA. The associations between total PFAS contaminant load with fish length for whole and fillet fish were evaluated using Pearson's or Spearman's rank correlations, where appropriate.

2.3.1. PFAS levels, capture location, and species

The associations between total PFAS, PFSA, PFCA, and PFOS levels in ng/g wet weight (ww) by whole fish or fillet within geographic location (AR, CR, or CH), and by species were determined using a series of linear regression models. Comparisons of contaminant levels between geographic locations and between fish species were conducted using linear contrasts from the models. Contaminant levels were natural log transformed to meet model assumptions. Given the limited sample size, pairwise comparisons were considered for any model with an overall $p < 0.20$. Additionally, for pairwise comparisons we considered both the unadjusted p-value and the Tukey's HSD (honest significant difference) adjusted p-value. Due to the small sample size for each species within a geographic location, we did not consider models with additional covariates (e.g. fish length) or a location by species interaction. Thus, we did not conduct formal hypothesis testing to compare differences in contaminant loading for species within location or location by species. However, difference between species within location and differences between the same species across locations were examined graphically. Additionally, values below the limit of quantification (LOQ) were set at $LOQ/\sqrt{2}$ for calculating total PFAS, total PFSA, and total PFCA. A sensitivity analysis was performed in which we substituted 0 for all values below the LOQ to determine if there result for the statistical analyses changed.

2.3.2. Patterns in PFAS concentrations

We also evaluated the relative contribution of the different PFAS to total PFAS in whole fish or fillets to identify possible patterns of contaminant loads by fish species and location. Only contaminants for which at least 30% of samples had values above the LOQ were considered individually. Of the ten PFAS measured in each sample, PFOA, PFOS, PFNA, PFDA, and PFUnDA had detectable levels in at least 30% of samples. The relative amount of these 5 contaminants and the relative contribution of all other contaminants were calculated according to

$$\frac{\text{Specific contaminant} \frac{ng}{g\ ww}}{\text{Total PFAS} \frac{ng}{g\ ww}}$$

where the total PFAS includes all contaminants in a sample at or above the LOQ. Differences in the relative amounts of specific contaminant by species and by location were examined using a MANOVA approach. If the global MANOVA F-statistic was significant, differences in individual species or by location were examined for each component in the respective ANOVA model using pairwise comparisons with both the unadjusted p-value and the Tukey's HSD (honest significant difference) adjusted p-value.

2.3.3. Fish consumption/risk assessment

We also examined the contribution of fish consumption on human exposure to PFOS based on national and local fish consumption. The daily intake (DI) (Eq. (1)) is the average daily consumption of fish (DC fish) per unit body weight in g/kg/day is based on the average g ww/day of fish divided by average body weight (BW) kg, assuming an average body of 70 kg. The average daily intake (ADI) of PFOS (Eq. (2)) is then the concentration of PFOS in fish muscle tissue in ng/g ww (C_{PFOS}) times the DI. The hazard ratio (HR) (Eq. (3)) is the ratio of the ADI and the reference dose $(RfD)_{PFOS}$ and a HR > 1 indicates potential risk of human exposure.

$$DI = DC_{fish}/BW \quad (1)$$

$$ADI = C_{PFOS} \times DI \quad (2)$$

$$HR = ADI/RfD_{PFOS} \quad (3)$$

Values for daily fish consumption were selected based on the EPA estimates from NHANES data which found median total daily fish consumption in adults over 21 years of age residing along the Atlantic coast was 24.5 g/day (U.S. Environmental Protection Agency USEPA, 2014). Additionally, preliminary results of a survey given to licensed anglers from Berkeley and Charleston Counties in South Carolina examining consumption of the fish anglers caught found 30% of respondents consumed 1–2 fish meals per month, 20% consumed 3 fish meals per month, 24% consumed at 1 per week, 14% consumed 2 meals per week, and 6% consumed 3 or more fish means per week (Perkinson et al., 2016; J. Vena, unpublished data). Note only 5% of respondents never ate caught fish. Assuming an average fish meal is 8 oz. wet weight, anglers in Charleston and Berkley County consume between 16.2 g/day (two meals per month) and 97.2 g/day (3 meals per week).

3. Results and discussion

Data were collected from 39 whole fish and 37 fillets. Among whole fish, the number of fish from each capture location was similar with 15, 13 and 11 (38.5%, 33.3%, and 28.2%) of the samples coming from the CH, AR, and CR, respectively. Among fillets, 10 (27%) of the fish were captured in CH, 10 (27%) in the AR, and 17 (46%) in the CR. Both sample types were collected from croaker, mullet, red drum, spot and seatrout, whereas only fillets were collected from flounder. For fillets, the present study showed detection of 9 out of 11 PFASs (except PFHxS and PFHxA) were detected above the LOQ in at least one sample, while in whole fish 10 out of 11 PFASs (except PFDS) were detected above the LOQ. Biological data of fish collected, including length and weight, are shown in Supplemental Table 1. For both whole fish and fillets, there was no significant association between fish length or fish weight with total PFAS (data not shown). Studies reported that neither sex nor weight was a significant correlate of PFAS concentration in fish (Ye et al., 2008: Hoff et al., 2005) which suggests that for some species that bioaccumulation may be influenced by factors other than size in a variety to species. Bhavsar et al. (2016) reported that bioaccumulation factors for PFOS for individual fish species could vary by an order of magnitude even within a narrow size range as fish length and PFOS

Table 2A
Mean (SD) and median (range) PFAS levels (total PFAS, total PFSA, total PFCA, and PFOS) in whole fish in ng/g wet weight by capture location and by species. P-values at the top of each column are for one-way ANOVA of either location or species. Given the limited sample size, pairwise comparisons of all groups were evaluated if the p-value for the ANOVA model was $p < 0.2$.

Group	n	Total PFAS	Total PFSA	Total PFCA	PFOS
Location		0.441	0.281	0.240	0.352
Charleston Harbor	15	22.6 (15.3)	14.0 (10.3)	8.60 (5.46)	13.2 (10.1)
		19.2 (9.27, 67.8)	11.6 (3.51, 45.2)	5.77 (4.18, 22.7)	10.9 (2.99, 43.8)
Cooper River	11	26.7 (17.1)	16.4 (11.8)	10.4 (5.67)	15.3 (11.3)
		24.1 (6.20, 66.1)	15.7 (2.79, 43.1)	8.84 (3.40, 23.0)	14.3 (2.53, 41.5)
Ashley River	13	27.5 (18.6)	20.4 (16.4)	7.05 (3.00)	18.6 (15.2)
		21.2 (12.7, 85.4)	15.4 (8.94, 72.1)	6.76 (3.40, 13.3)	14.7 (7.83, 66.3)
Species		0.003	0.005	0.011	0.004
Mullet	9	12.7 (4.93)^{C,D,E}	6.80 (4.14)^{B,C,D,E}	5.94 (1.87)^{c,d}	6.23 (3.88)^{B,C,D,E}
		12.4 (6.20, 20.7)	5.03 (2.79, 14.4)	5.77 (3.40, 9.49)	4.77 (2.53, 13.7)
Spot	7	33.0 (17.7)	21.2 (11.4)	11.9 (6.68)	19.8 (11.5)
		28.4 (14.7, 67.8)	17.6 (11.0, 45.2)	10.8 (3.71, 22.65)	16.7 (9.46, 43.8)
Croaker	5	19.5 (2.57)	14.7 (3.05)	4.79 (0.8)^{c,D,e}	13.7 (2.68)
		20.6 (15.2, 21.3)	15.4 (9.96, 17.8)	4.78 (3.28, 5.17)	14.8 (9.37, 16.3)
Red Drum	9	29.6 (16.6)	18.7 (11.1)	10.9 (6.21)	17.6 (10.8)
		27.0 (11.3, 66.1)	17.5 (7.12, 43.1)	10.6 (4.18, 23.0)	16.5 (6.41, 41.5)
Seatrout	9	31.1 (21.6)	22.7 (19.5)	8.44 (2.39)	20.7 (18.0)
		23.4 (17.3, 85.4)	14.2 (10.2, 72.1)	8.37 (5.70, 13.3)	13.2 (9.25, 66.3)

a: Unadjusted $p < 0.05$ compared to Ashley River; A: Adjusted and unadjusted $p < 0.05$ compared to Ashley River.
b: Unadjusted $p < 0.05$ compared to Croaker; B: Adjusted and unadjusted $p < 0.05$ compared to Croaker.
c: Unadjusted $p < 0.05$ compared to Red Drum; C: Adjusted and unadjusted $p < 0.05$ compared to Red Drum.
d: Unadjusted $p < 0.05$ compared to Spot; D: Adjusted and unadjusted $p < 0.05$ compared to Spot.
e: Unadjusted $p < 0.05$ compared to Seatrout; E: Adjusted and unadjusted $p < 0.05$ compared to Sea trout.
f: Unadjusted $p < 0.05$ compared to Flounder; F: Adjusted and unadjusted $p < 0.05$ compared to Flounder.

typically lack a positive relationship. In contrast, a positive relationship between PFOS concentration and fish length was observed in carp (Gewurtz et al., 2014) and weight in tilapia (Pan et al., 2014) and trout (Furdui et al., 2007).

3.1. PFAS levels in whole fish and fillets

The overall summary of PFASs is provided in Table 2A for whole fish and in Table 2B for fillets. Mean total PFAS, PFSA, PFCA, and PFOS were lower in fillets relative to whole fish. Within species pooled across locations, significantly lower concentrations of total PFAS, PFSA, PFCA, and PFOS were found in fillets relative to whole fish for mullet and seatrout. Fillets also had significantly lower levels of total PFAS, PFCA, and PFOS relative to whole fish in red drum. Croaker and spot only exhibited significant differences in PFCA for fillets versus whole fish. For whole fish, mean concentrations for total PFAS (ng/g ww) ranged

Table 2B
Mean (SD) and median (range) PFAS levels (total PFAS, total PFSA, total PFCA, and PFOS) in fillets in ng/g wet weight by capture location and by species. P-values at the top of each column are for one-way ANOVA of either location or species. Given the limited sample size, pairwise comparisons of all groups were evaluated if the p-value for the ANOVA model was $p < 0.2$.

Group	n	Total PFAS	Total PFSA	Total PFCA	PFOS
Location		0.056	0.042	0.419	0.036
Charleston Harbor	10	10.3 (5.95)^{a}	6.99 (5.29)^{a}	3.28 (1.24)	6.44 (5.54)^{A}
		9.19 (2.99, 22.8)	5.57 (0.68, 17.1)	3.29 (1.53, 5.74)	5.17 (0.43, 16.8)
Cooper River	17	14.9 (8.09)	10.3 (6.29)	4.63 (2.72)	9.24 (6.54)^{a}
		16.7 (3.35, 29.5)	9.85 (1.29, 21.6)	3.87 (2.06, 11.4)	9.45 (0.43, 20.5)
Ashley River	10	20.0 (10.0)	16.2 (9.40)	3.81 (1.00)	15.5 (9.18)
		22.9 (3.30, 36.7)	18.9 (0.74, 30.9)	3.51 (2.56, 5.77)	18.3 (0.43, 30.0)
Species		< 0.001	< 0.001	< 0.001	< 0.001
Mullet	9	5.58 (2.84)^{b,C,D,E,F}	2.88 (2.68)^{B,C,D,E,F}	2.70 (0.72)^{c,D,F}	1.40 (1.48)^{B,C,D,E,F}
		4.78 (2.99, 11.4)	1.92 (0.68, 9.04)	2.38 (2.07, 4.27)	0.43 (0.43, 4.81)
Spot	6	19.9 (5.02)	14.1 (4.49)	5.77 (2.54)	13.5 (4.32)
		19.0 (13.7, 27.9)	12.9 (9.85, 21.6)	5.38 (3.41, 10.4)	12.0 (9.45, 20.5)
Croaker	2	13.6 (1.87)	11.1 (0.49)	2.51 (1.38)^{c,D,F}	10.8 (0.40)
		13.6 (12.3, 15.0)	11.1 (10.8, 11.5)	2.51 (1.53, 3.48)	10.8 (10.5, 11.1)
Red Drum	5	13.3 (6.36)^{f}	9.13 (6.30)f	4.24 (0.98)	8.62 (6.09)^{f}
		10.6 (7.81, 23.5)	7.40 (3.70, 19.7)	4.11 (3.17, 5.83)	7.02 (3.24, 18.8)
Seatrout	6	12.7 (8.25)^{d,F}	9.88 (7.95)f	2.85 (0.60)^{c,D,F}	9.25 (7.90)^{f}
		9.94 (4.03, 23.5)	6.93 (2.15, 20.8)	2.80 (1.88, 3.65)	6.30 (1.45, 20.0)
Flounder	9	24.1 (6.28)	18.8 (5.74)	5.27 (2.45)	18.1 (5.53)
		23.5 (16.9, 36.7)	18.3 (11.8, 30.9)	4.86 (3.24, 11.4)	17.7 (11.3, 30.0)

a: Unadjusted $p < 0.05$ compared to Ashley River; A: Adjusted and unadjusted $p < 0.05$ compared to Ashley River.
b: Unadjusted $p < 0.05$ compared to Croaker; B: Adjusted and unadjusted $p < 0.05$ compared to Croaker.
c: Unadjusted $p < 0.05$ compared to Red Drum; C: Adjusted and unadjusted $p < 0.05$ compared to Red Drum.
d: Unadjusted $p < 0.05$ compared to Spot; D: Adjusted and unadjusted $p < 0.05$ compared to Spot.
e: Unadjusted $p < 0.05$ compared to Seatrout; E: Adjusted and unadjusted $p < 0.05$ compared to Seatrout.
f: Unadjusted $p < 0.05$ compared to Flounder; F: Adjusted and unadjusted $p < 0.05$ compared to Flounder.

from 12.7 in mullet to 33.0 in spot with the highest individual level of 85.4 in seatrout. For fillets, mean total PFAS levels ranged from 5.58 in mullet to 19.9 in spot with the lowest individual level of 2.99 in mullet and the highest individual level of 23.5 in both red drum and seatrout. Additionally, flounder were also collected for fillets which had the highest mean total PFAS levels (24.1; range 16.9–36.7 ng/g ww) than any other fish species.

Many studies have focused on PFAS concentrations in whole fish and liver (Senthilkumar et al., 2007; Hart et al., 2008; Ye et al., 2008). From an ecological perspective whole fish and liver data can inform on levels consumed by piscivorous predators such as dolphins. However, to assess human PFAS exposure through the consumption of contaminated fish information is needed on the relationships between PFAS levels in liver, whole body and fillets. Recently, both Fliedner et al. (2018) and Mazzoni et al. (2019) reported PFAS concentrations in both fillets and whole fish and relationship between the two values. Considering that studies have indicated that people with high intake of fish especially sport caught fish in their diet had higher blood PFAS concentrations (Falandysz et al., 2006; Bloom et al., 2009; Hölzer et al., 2011) and even low levels of seafood have been associated with elevated PFAS levels (Christensen et al., 2017), it is important to assess risk of exposure to these compounds. In the present study we measured both whole fish and fillets for PFASs. Although PFAS measurements on fillets were not possible from the same fish processed as whole fish, they were collected in the same locations, so such comparisons are useful.

For all fish species, total PFAS concentrations were higher in whole fish compared to fillets by a factor of 1.4–2.5 (2.3X in mullet, 1.7X in spot, 1.4X in croaker, 2.2X in red drum and 2.5X in seatrout). PFOS fish to fillet ratios ranged from 1.3 to 2.3 with exception of mullet with 4.5 which is consistent with ratios found by Fliedner et al. (2018). Highest concentrations of PFOS and total PFAS are typically found in fish blood, followed by liver, brain, and muscle, supporting PFOS can bind more easily to serum proteins than to fatty tissues (Shi et al., 2012). Of all fish tissues examined in mullet by Shi et al. (2012), liver had the highest PFOS concentrations (192 ng/g ww) and gonad levels were 80.2 ng/g while muscle contained 9.01 ng/g (Bangma et al., 2018). Hence, it is not surprising that higher PFAS levels found in our study were in whole fish since they contain organs compared to fillets with only muscle tissue.

Levels of PFAS vary in biota and the longer-chained PFAS are considered bioaccumulative (Conder et al., 2008). The PFAS commonly found in the highest concentration in biota is PFOS with the highest bioaccumulation potential in food webs and thus, the highest PFOS values occur in top carnivores and in urban rivers (Houde et al., 2011). Whole fish homogenates from Ohio, Missouri, and Mississippi Rivers had PFOS levels that contributed more than 80% of the total PFAS composition in fish with mean concentrations of 84.7, 147 and 93.1 ng/g ww, respectively (Ye et al., 2008). Similarly, in our study, PFOS was also the dominant PFAS compound in whole fish representing 45.9% in mullet, 58.5% in red drum, 59.7% in spot, 62.4% in seatrout and 69.6% in croaker (Table 3). In fillets, PFOS was also the highest congener with similar results found in whole fish (59.7% in red drum, 62.9% in seatrout, 67.8% in spot, 79.9% in croaker) but with only 25.5% in mullet. In fillets of flounder PFOS represented 75% of total PFAS.

PFOS has been detected in fish globally with concentrations varying among locations ranging from a few to hundreds ng/g level. In northern Germany, edible fish samples caught from densely populated regions were reported at levels between 8.2 and 225 ng/g ww while samples from marine or remote locations had lower non-detectable levels (Schuetze et al., 2010). PFOS concentrations in fish from two Baltic regions (medians 2.9–12 ng g ww and 1.0–2.5 ng g ww) were also higher in the site near an urban area (Berger et al., 2009). Some of the highest concentrations have been reported in fish sampled in 2008 from the Mississippi River (28.5–382 ng/g muscle) (Malinsky et al., 2011) and also in fish collected in 2007 from the Minnesota Rivers (ranging up to 2000 ng/g ww) adjacent to the 3 M Company, one of the former largest fluorochemical plants (Delinsky et al., 2010). Lower levels have been reported in China where muscle PFOS concentrations ranged from 0.3 to 13.9 ng/g ww (Gulkowska et al., 2006) and in fish from Italian subalpine lakes averaging 3.1 ng/g (Mazzoni et al., 2019).

Generally, in our study the concentration of PFOS in fish muscle (1.52–29.97 ng/g ww) and whole fish (2.53–66.33 ng/g ww) were comparable or lower than other areas in the U.S. Data from the U.S. National fish tissue monitoring indicate widespread occurrence of many PFAS, with PFOS being the most predominant, in the Great Lakes and in urban rivers across the country (Stahl et al., 2014). In the Great Lakes 100% of the fish contained some detectable PFAS and the median PFOS levels in fillets was 10.7 ng/g ww for urban rivers, with a maximum of 127 ng/g ww (Sinclair et al., 2006). For lake trout (*Salvelinus namaycush*), Gewurtz et al. (2013) found mean PFOS levels in whole fish 90 and 62 ng/g ww in fish from Lake Erie and Lake Ontario, respectively, whereas levels were lower in fish from Lake Superior and systems located in northern Canada, Pacific, and Atlantic regions. PFOS in muscle of striped mullet (*Mugil cephalus*) at Merritt Island National Wildlife Refuge in Florida had mean PFOS levels of 9.01 ng/g (Bangma et al., 2018) compared to our study, which had a median of 1.48 ng/g ww for the same species in Charleston. Differences in PFOS concentrations observed in fish are likely a function of the location and the different species, as concentrations of PFOS and other PFASs in fish are generally not related to indicators of food web structure or diet such as stable isotopes of nitrogen and carbon, or fish lipid, size, age, and growth (Guo et al., 2012). In the present study, a number of factors may contribute to the PFASs concentrations in the fish species examined such as PFAS concentrations in water/sediment, and species-specific habitats and feeding/dietary characteristics, bioaccumulation and elimination pathways. Previous studies indicate that PFAS levels are higher in piscivorous fishes (Kannan et al., 2005; Martin et al., 2004) demonstrating biomagnification in food webs. However, lower trophic level fish species, such as carp, may nevertheless accumulate substantial PFAS concentrations that require consumption advisories (Ye et al., 2008). It has been suggested that concentrations of PFOS and exposure to them may be greater in the water column than sediment (Tomy et al., 2004) which would explain why mullet, a benthic herbivore (Wenner et al., 1990) had the lowest PFAS levels compared to higher trophic level species such as red drum and seatrout.

The highest PFAS concentrations in fish have typically been observed in densely populated urban regions. The concentrations of PFOS in a variety of habitats in the Canadian environment were generally higher in heavily populated urban and industrialized locations, especially in southern Ontario, than in more remote locations. Consistent with other studies conducted throughout the world (Houde et al., 2006, 2011; Suja et al., 2009), this pattern indicates that activities associated with human population, such as the use and disposal of PFAS containing consumer products, continue to be important sources of PFASs to the Canadian environment. While the highest PFAS concentrations have been found near direct discharges from industries using PFASs, these chemicals are also found in air, sediment and biota in the Arctic, which lack direct sources (Lindstrom et al., 2011). Aquatic environments tend to be the primary sink in the environment for long chain PFASs. Sources, both point and nonpoint, into the aqueous environment include industrial or municipal wastewater treatment plants, atmospheric deposition and fill leachate, and soil/street surface runoff (Ahrens, 2011). A previous study examining PFASs in sediment from our study system reported higher levels in the AR and CR compared to CH (White et al., 2015), which also reflects the findings of PFAS in fish from this study. Multiple hot spots were found in surface sediments samples likely due to inputs from the rivers and creeks as well as potential point sources reflecting the relative high population density of Charleston and amount of industrial activity.

Table 3
Average relative percent of total PFAS for PFOSA, PFOS, PFNA, PFDA, and PFUnA by sample type, by location or species within whole fish and by fillets. The global P-value in the last column is for the overall MANOVA and P-values at the top of each column are for one-way ANOVA of either sample type, location, or species.

Sample		n	PFOSA	PFOS	PFNA	PFDA	PFUnA	Global p
All	Type		0.354	0.981	0.011	0.877	< 0.001	< 0.001
	Whole	39	3.42	58.2	3.01	13.5	12.1	
	Fillet	37	5.36	58.0	5.38	13.3	6.34	
Whole	Location		0.050	0.013	< 0.001	< 0.001	0.150	< 0.001
	Charleston Harbor	15	2.54[a]	55.8[a]	4.43[A]	14.3[A]	11.2	
	Cooper River	11	2.94	52.9[a]	2.96	16.2[A]	16.3	
	Ashley River	13	4.83	65.2	1.69	10.3	9.5	
	Species		0.314	< 0.001	0.901	0.116	< 0.001	0.0198
	Mullet	9	2.04	45.9[B,c,d,E]	2.86	15	21.7[B,D,E]	
	Spot	7	3.81	59.7	3.13	14.7	8.24	
	Croaker	5	3.64	69.6	2.49	9.31	4.97	
	Red Drum	9	3.12	58.5	3.2	13.8	13.3	
	Seatrout	9	4.66	62.4	3.56	13.1	8.21	
Fillet	Location		0.623	0.234	0.550	0.136	0.097	0.402
	Charleston Harbor	10	5.14	52.1	5.34	15.8	6.08	
	Cooper River	17	7.30	55.1	6.22	13.8	7.68	
	Ashley River	10	2.23	69.0	3.96	9.97	4.33	
	Species		0.286	< 0.001	0.042	0.238	0.094	< 0.001
	Mullet	9	1.44	25.5[B,C,D,E,F]	9.47[d,f]	17.7	8.53	
	Spot	6	2.03	67.8	2.6	12.3	5.86	
	Croaker	2	0.70	79.9	4.91	9.04	1.39	
	Red Drum	5	2.18	59.7	6.53	14.6	8.52	
	Seatrout	6	4.65	62.9	5.59	12.4	4.73	
	Flounder	9	1.79	75	2.48	10.4	5.43	

a: Unadjusted p < 0.05 compared to Ashley River; A: Adjusted and unadjusted p < 0.05 compared to Ashley River.
b: Unadjusted p < 0.05 compared to Croaker; B: Adjusted and unadjusted p < 0.05 compared to Croaker.
c: Unadjusted p < 0.05 compared to Red Drum; C: Adjusted and unadjusted p < 0.05 compared to Red Drum.
d: Unadjusted p < 0.05 compared to Spot; D: Adjusted and unadjusted p < 0.05 compared to Spot.
e: Unadjusted p < 0.05 compared to Seatrout; E: Adjusted and unadjusted p < 0.05 compared to Seatrout.
f: Unadjusted p < 0.05 compared to Flounder; F: Adjusted and unadjusted p < 0.05 compared to Flounder.

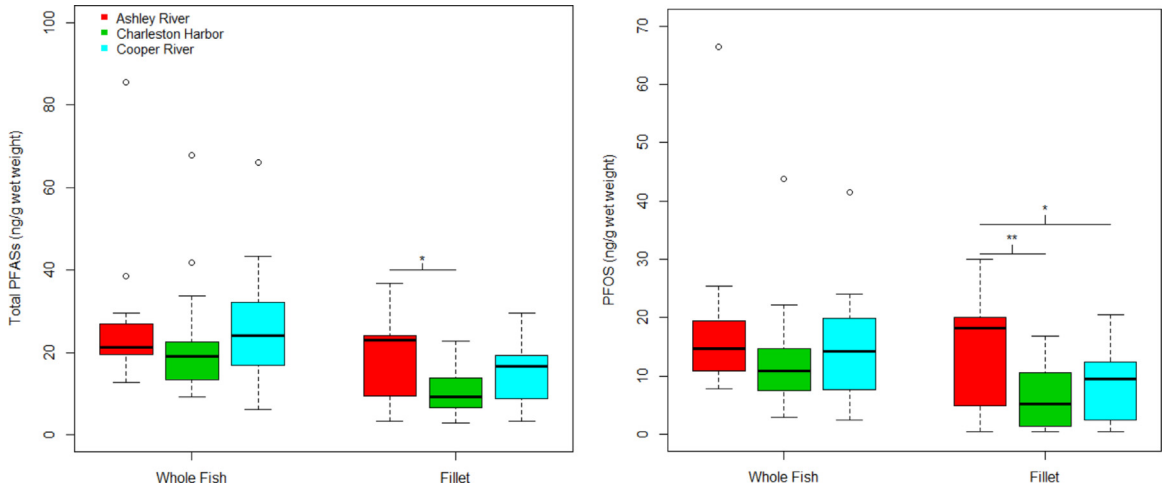

Fig. 2. Distribution of (A) Total PFAS and (B) PFOS in ng/g wet weight by location (whole fish vs. fillet). Boxes represent the 25th 50th and 75th percentiles for the observed distribution, the whiskers represent 1.5 × inner-quartile range (IQR), and points outside the whiskers represent any observed value that is less than or greater than 1.5 × IQR. The number of samples per group are shown below each box. Note, lines above the boxes indicate significant relationships; with * representing significance at p < 0.05 for the unadjusted p-values and ** representing significance at p < 0.05 for the Tukey HSD adjusted p-value.

3.2. Relative levels of PFAS in whole fish and fillets

The relative amounts of specific of PFAS, presented as the percent of total PFAS amount in ng/g ww, for each whole fish or fillet are presented in Supplemental Fig. 1A and B respectively. Specific PFAS considered included PFOSA, PFOS, PFNA, PFDA, PFUnA, and other (comprising all remaining PFAS). Mean relative percent of each PFAS by sample type, by location or species within whole fish and by fillets are shown in Table 3. Relative amounts of PFOS, PFOSA, and PFDA were similar between whole fish and fillets. However, whole fish had significantly higher relative amounts of PFUnA and lower relative amounts of PFNA compared to fillets. In whole fish there were notable differences in the relative amount of PFOS, PFNA, and PFDA by capture location suggestive of potential source differences. Specifically, fish from the AR had significantly greater relative amounts of PFOS and lower relative amounts of PFDA compared to fish from the CR and CH. Additionally, whole fish from the AR had lower relative amounts of PFNA compared to fish from the CH. Mullet also had significantly lower relative amounts of PFOS on average compared to all other species and higher relative amounts of PFUnA compared to croaker, seatrout, and spot.

There were not notable differences in the relative amount of any of

the PFAS by capture location in fillets. However, similar to whole fish, mullet fillets had significantly lower relative PFOS levels compared to all other species. Unlike whole fish samples, mullet fillets had significantly higher PFNA levels on average compared to spot and flounder. In our study the following concentrations of PFAS compounds occurred in decreasing order in all species, except mullet, processed either as whole or fillets as follows: PFDA > PFUnA > PFNA. In mullet, whole fish had PFUnA > PFDA > PFNA and fillets had PFDA > PFNA > PFUnA. Possible explanation for the differences in the relative distribution of individual PFAS in mullet with higher PFUnA in whole fish may relate to its benthic habitat and greater exposure to this compound which was relatively abundant in local sediments (White et al., 2015).

3.3. Comparison of PFASs in fish species and locations

Total PFAS and PFCA for fish captured in the CR were significantly lower in fillets relative to whole fish (Fig. 2). Only PFCAs were significantly lower in fillets compared to whole fish for samples from the AR. In whole fish samples, there were differences in total PFASs, total PFSAs, total PFCAs, and PFOS between different fish species (Table 3A). Specifically, mullet had significantly lower levels of total PFAS, PFSA, PFCA and PFOS compared to red drum and spot and these differences remained significant after adjusting for multiple comparisons for PFAS, PFSA, and PFOS (Fig. 3). Mullet also had significantly lower levels of total PFAS, PFSA, and PFOS compared to seatrout and lower levels of PFSA and PFOS compared to croaker, even after adjusting for multiple comparisons. There were no significant differences in PFAS levels in whole fish between the three capture locations. Generally, total PFSAs concentrations were greater than total PFCA levels in all fish species either processed as whole or fillets. The relative abundance of PFCAs in whole fish were 1–3 times greater than PFSAs in all species, with mullet having the lowest and croaker the highest amount. The highest concentration of total PFCAs (sum of C8-C15) was observed in spot. For location, PFSAs were 3X higher in all than PFCAs in all fish from CH and 1.6 fold-higher in fish from the rivers.

Differences observed between species were similar in fillets as reported in whole fish (Table 2B). Similar to whole fish samples, mullet had significantly lower levels of total PFAS, PFSA, and PFOS compared to all other species and these differences remained significant after adjusting for multiple comparisons. Mullet also had significantly lower levels of PFCA compared to red drum, spot and flounder and differences remained significant for spot and flounder after adjusting for multiple comparisons. Red drum and seatrout also exhibited lower levels of total PFAS and PFOS relative to flounder. Seatrout also had lower levels of PFAS and PFCA compared to spot. Croaker had lower levels of PFCA compared to red drum, spot, and flounder. Unlike whole fish, there were notable differences in contaminant levels by capture location. Specifically, fillets from fish captured in the AR had significantly higher levels of PFSA and PFOS compared to both the CR and CH fish and significantly higher total PFAS levels relative to fish captures in the CH. Interestingly, while no differences in PFAS levels occurred in whole fish by capture location, fillets had differences that were dependent upon catch location. In contrast to lipophilic organic pollutants such as PCBs, the binding of PFOS to liver and blood proteins contributes to the higher levels found in whole fish. As to why location differences in PFAS levels occurred only in fillets may be related to PFAS found only in muscle tissue versus PFAS in homogenized fish tissues.

3.4. Human exposure/risk assessment

As demonstrated in numerous studies, fish consumption is a major pathway for human exposure to PFASs. National risk-based consumption limits or screening values for human health have not yet been developed for PFAS in the U.S. To assess the potential health risks to humans we examined dietary exposure of PFOS levels in fish fillets to Fish Consumption Screening Values (FCSV) from the Michigan Fish Consumption Advisory Program for PFOS (Michigan Department of Health and Human Services, 2016). Michigan's FCSV values, one of the only regulations on PFOS fish consumption in fish tissue in the U.S., serve as guidelines for the general public to determine how often PFOS containing fish should be consumed. Fig. 4 shows PFOS levels in fish muscle for all species and locations compared to total length of fish and against meals per month. Since PFOS was the predominant PFAS in fish collected from this region, risk assessment was only performed for PFOS. Results indicate that several fish were within the four meals per month guidelines, mostly flounder and seatrout that exceeded 19 ng/g ww. None of the fish fell into the 'Do Not Eat' category. Similar comparisons in mullet muscle from two sites in Florida ranged within 16 meals per month to once a month with stricter categories for one site (Bangma et al., 2018).

Based on national fish consumption data, the ADI for fish species in the present study ranged from 2.21 to 6.20 ng/kg/day (data not shown). Human dietary intake of PFOS through fish consumption varies in regions of the world. Shi et al. (2012) estimated ADI at 0.24 ng/kg/d for PFOS in farmed freshwater fish in Beijing, China indicating a low

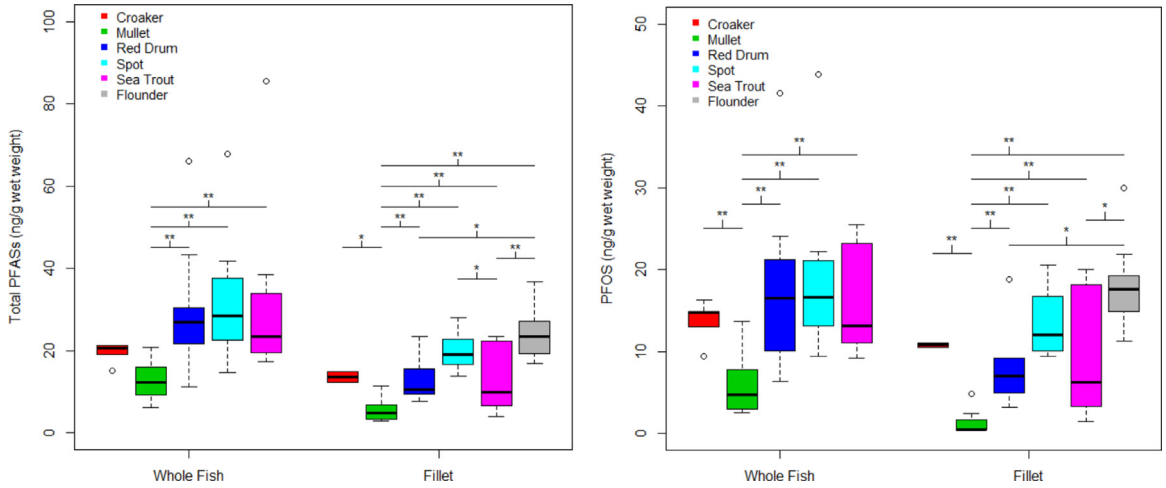

Fig. 3. Distribution of (A) Total PFAS and (B) PFOS in ng/g wet weight by species (whole fish vs. fillet). Boxes represent the 25th 50th and 75th percentiles for the observed distribution, the whiskers represent 1.5 × inner-quartile range (IQR), and points outside the whiskers represent any observed value that is less than or greater than 1.5 × IQR. The number of samples per group are shown below each box. Note, lines above the boxes indicate significant relationships; with * representing significance at $p < 0.05$ for the unadjusted p-values and ** representing significance at $p < 0.05$ for the Tukey HSD adjusted p-value.

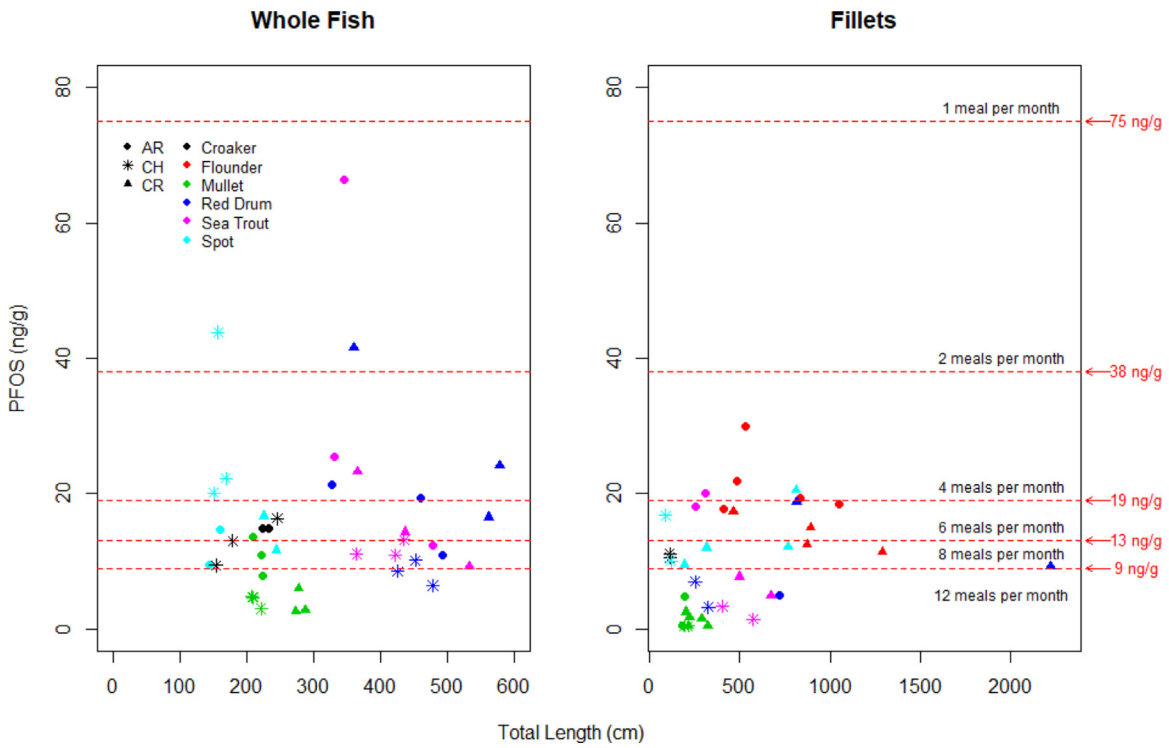

Fig. 4. Comparison of PFOS levels versus total length in fillets. Fish Consumption Screening Values (FCSV) from Michigan Department of Health and Human Services (2016).

health risk posed from PFASs to the residents of Beijing through the consumption of fish. Seafood from fish markets in two coastal cities in China ranged from 1.7 to 2.8 ng/kg bw/day (Gulkowska et al., 2006). The ADI for PFOS in ng/kg/d averaged 2.4 in the Hong Kong population (Zhou et al., 2014), 0.62 in Sweden (Berger et al., 2009) and 0.78 in Norway (Haug et al., 2010). Higher ADI were reported for 9 freshwater species in China averaging 31 ng/kg/d and ranging from 1.2 to 69 ng/kg/d (Pan et al., 2014), reflecting both high PFOS levels and relatively high fish consumption rates. To assess potential impacts on human health resulting from consumption of contaminated fish a risk assessment based on tolerable daily intake (TDI) is needed. Currently there is no standardized national or international TDI for PFOS or PFOA. The European Food Safety Authority's Scientific Panel (2008) on contaminants in the food chain (CONTAM) identified 30 μg/kg body weight per day as NOAEL effect to derive provisional TDI 150 ng/kg per day with overall uncertainty factor of 200 to NOAEL (European Food Safety Authority EFSA, 2008). This value would be exceeded for an adult with body wt 60 kg when consuming fish that contains 30 μg/kg of PFOS and consumption of 300 g fish per day.

The RfD is an estimate of the daily exposure level that is likely to be without harmful effects over a lifetime. When HR > 1, it indicates that there is potential risk of human exposure to PFASs, otherwise, there is a low potential health hazard. Provisional references doses (RfD) for PFOS have been suggested as 0.025 μg/kg/day (25 ng/kg bw/day) on the basis of rat multigenerational studies (Thayer, 2002). EPA derived oral non-cancer reference doses (RfDs) of 0.00002 mg/kg/day (0.02 μg/kg) for both PFOS and PFOA (U.S. Environmental Protection Agency USEPA, 2016). Table 4 shows the mean HRs for filleted samples by capture location and species based on different amount of fish consumed per unit time. For all risk assessment analyses, we assumed a conservative reference dose of 25 ng/kg body weight/day. Compared to the average daily fish intake of 24.5 g/day estimated for people living in Atlantic coastal areas in the US (U.S. Environmental Protection Agency USEPA, 2014), none of the fillets in our study had sufficiently high levels of PFOS to cause increased risk as defined by a HR > 1.

Applying results of a recent study of fish consumption habits of recreational fishermen in Charleston and Berkley counties in South Carolina, which corresponds to our capture locations as referred above (Perkinson et al., 2016; J. Vena, unpublished data), 8 of the 37 fillets in our study had HRs > 1 based on the PFOS levels found in the samples and 1 of the 37 samples had elevated hazard if more than 2 fish meals per week were consumed. None of the samples had elevated hazard if 1 or fewer fish meals per week are consumed (Table 4). Fish from the AR had the highest corresponding HRs with 6 of the 10 fish captures in the AR having HR > 1 compared to 2 of 17 from the CR and 0 of 10 from the CH if 3 or more fish meals are consumed per week. We also examined HRs based on PFOS levels by fish species. Flounder was the species most likely to have HR > 1 although several additional species including seatrout, spot and red drum showed some evidence of potentially elevated hazards. Specifically, 4 of the 9 flounder fillets, 2 of the 6 seatrout fillets, 1 of the 6 spot fillets and 1 of the 5 red drum fillets had sufficiently high PFOS levels to yield an increased hazard if consumed 3 or more times per week. Only 1 of the 9 flounder fillets and no other samples had HR > 1 when 2 fish meals per week are consumed. Mullet had little impact on HRs in all capture locations with the highest estimated HR for 3 meals per week of only 0.27.

Also, we assessed the maximum allowable number of 8 oz. fish meals per month one could consume based on the contaminant load in each fillet. The maximum number of fish meals per month is defined as the number of 8 oz. fish meals a 70 kg person could eat per month and still have a HR less than 1, indicating no risk from eating this number of meals. Values were truncated at 40 meals per month thus fish for which a larger maximum allowable meals per month was greater than 40 were set to 40, which included 5 of the 9 mullet fillets. The maximum allowable number of meals per week was greater than 1 for all samples with a minimum of 1.8. The maximum allowable meals per month by species and capture location are provided in Fig. 4. Another study calculated HRs for fish using a RfD for PFOS of 0.025 μg/g/d and reported 0.11 and 0.07 in China (Gulkowska et al., 2006). Freshwater fish from rivers in the Pearl River, South China ranged from 0.05 to 2.9 with 4 out of 9 species having HR values more than 1.0 indicating a health risk for the 4 fish species (Pan et al., 2014). In our study, flounder had a

Table 4
Median (min, max) hazard ratios (HRs) by capture location and fish species for (a) EPA estimates of daily fish intake from NHANES of 24.5 g/day and (b) by number of 8 oz. fish meals per week for a 70 kg individual. All estimated HRs assume a reference dose of 25 ng PFOS/kg BW/day. Note that 3 meals/week corresponds to 97.2 g daily fish intake, 2 meals/week to 64.8 g daily fish intake, 1 meal/week to 32.4 g daily fish intake, and 0.5 g meals/week to 16.2 g daily fish intake.

	NHANES (24.5 g/day)	3 meals/wk	2 meals/wk	1 meals/wk	0.5 meals/wk
Location					
Ashley River	**0.26** (0.01, 0.42)	**1.01** (0.02, 1.66)	**0.68** (0.02, 1.11)	**0.34** (0.01, 0.55)	**0.17** (0.00, 0.28)
Cooper River	**0.13** (0.01, 0.29)	**0.52** (0.02, 1.14)	**0.35** (0.02, 0.76)	**0.17** (0.01, 0.38)	**0.09** (0.00, 0.19)
Charleston Harbor	**0.07** (0.01, 0.24)	**0.29** (0.02, 0.93)	**0.19** (0.02, 0.62)	**0.10** (0.01, 0.31)	**0.05** (0.00, 0.16)
Species					
Croaker[a]	0.15, 0.16	0.59, 0.62	0.39, 0.41	0.20, 0.21	0.10, 0.10
Flounder	**0.25** (0.16, 0.42)	**0.98** (0.63, 1.66)	**0.65** (0.42, 1.11)	**0.33** (0.21, 0.55)	**0.16** (0.10, 0.28)
Mullet	**0.01** (0.01, 0.67)	**0.02** (0.02, 0.27)	**0.02** (0.02, 0.18)	**0.01** (0.01, 0.09)	**0.00** (0.00, 0.04)
Red Drum	**0.10** (0.05, 0.26)	**0.39** (0.18, 1.04)	**0.26** (0.12, 0.69)	**0.13** (0.06, 0.35)	**0.06** (0.03, 0.17)
Spot	**0.17** (0.13, 0.29)	**0.67** (0.52, 1.14)	**0.45** (0.35, 0.76)	**0.22** (0.17, 0.38)	**0.11** (0.09, 0.19)
Seatrout	**0.09** (0.02, 0.28)	**0.35** (0.08, 1.11)	**0.23** (0.05, 0.74)	**0.12** (0.03, 0.37)	**0.06** (0.01, 0.19)

[a] There were only 2 croaker fillet samples thus the two values are presented rather than the median (min, max).

median HR value of 1.0 for the category of 3 meals/per week as shown in Table 4. However, the maximum HR values exceed 1.0 for flounder, red drum, spot, and seatrout. Additionally, the maximum HR for flounder also exceeded 1.0 for the category of 2 meals/week.

The recreational fishing community often eat large quantities of fish from a few local sources. It has been found that even where state consumption advisories are in place, anglers are often unaware of these advisories or choose to ignore them (Beehler et al., 2001; May and Burger, 1996). These anglers may be at greater risk from specific polluted areas and also species of fish (May and Burger, 1996; Beehler et al., 2001). Demographics at greater exposure risk may include pregnant women, sport-anglers, subsistence anglers, etc. One such group, the Gullah/Geechee (G/G) is a unique national ethnic group that lives along the intercoastal waterway of the southeast with approximately 1 million people. As urban sprawl spreads across the region, potential health risks to the G/G population also increase since they are "subsistence" users of these watersheds (Spruill et al., 2013). The G/G population of Coastal Carolina have elevated levels of PFASs, which has been associated with markers of autoimmunity (Miller et al., 2012). Similar to other African American communities, Gullah women are disproportionately affected by systemic lupus erythematosus (Lim et al., 2014). With local seafood consumption being a dietary staple, potential pollutant contamination of the seafood is of high concern to the health of the Gullah community (Spruill et al., 2013). In parallel, bottlenose dolphins' resident to coastal estuarine areas of Charleston with high site fidelity are among marine mammals with the highest PFASs and associated immune and other detrimental health effects, highlighting the hazardous nature of these chemicals (Fair et al., 2013; Houde et al., 2005). Although we know dolphins' resident to Charleston accumulate extremely high levels of these chemicals we know little about exposures in humans living adjacent to these areas, and particularly regarding consumption of local seafood.

Fish is one of the highest sources of PFASs as well as other POPs. Thus, while the current PFAS levels in fish species from the Charleston may not pose risks for PFOS exposure until higher consumption levels of 2 or 3 meals a week are attained for flounder, red drum, spot, and seatrout, it should be noted that people are exposed to multiple contaminants such as PCBs, pesticides and flame retardants in fish as indicated in our previous study in these same fish (Fair et al., 2018). About half of the advisories presently issued are potentially not adequately protective in Great Lakes since they do not consider additive effects of mixtures of chemicals (Gandhi et al., 2017). The ability to assess risk imposed by consumption of these fish is limited since RfD is available for only PFOS and highlighting the need for RfD's on other PFAS compounds to inform on additive effects from other congeners as well as other chemicals. More stringent advisories may be warranted due to multiple contaminant exposures and the complex mixture of contaminants found in fish. Due to the small sample size both in whole fish and fillets for each species within a geographic location, our data analysis of PFAS levels by fish species and by geographic location were conducted separately; therefore, our interpretation has some limitations as to whether the differences found are conclusive specifically to location or species or the combination of the two. Additionally, the limited sample size precluded controlling for other covariates such as fish length or weight when examining differences in PFAS levels between species or geographic locations.

3.4.1. *Wildlife risk assessment*

Limited information exists for assessing the risk of PFASs for wildlife. We used Canada's recent Federal Environmental Quality Guidelines (FEQGs) to provide a benchmark for the hazard associated with PFOS for fish health which is listed for fish tissue as 9.4 mg/kg ww (Environment Canada and Climate Change Canada ECCC, 2018). The Federal Fish Guideline (FFTG) benchmark allows for protection of fish from the direct adverse effects of bioaccumulated contaminants such as PFOS. In the present study, all of the fish were well below this level which suggest no potential risk to fish health in their collection location. The Federal Wildlife Dietary Guidelines (FWiDGs) developed by Canada also provides benchmarks for concentrations of toxic substances that are consumed by wildlife in order to protect aquatic and terrestrial biota, The FEQG for PFOS in mammalian wildlife diet is 4.6 μg/kg ww food. Using this value, the PFOS levels in whole fish in the present study exceeded the FEQG for the protection of mammals that eat fish in all samples with the exception of three fish. This finding suggests that this compound could represent a potential risk to wildlife predators such as dolphins. Since FEQGs are preventive, and not predictive, wildlife population health assessments would be necessary to determine whether negative impacts are actually occurring. Dolphins are relevant sentinels for examining the accumulation and potential health impacts of persistent environmental chemicals such as PFASs due to their high trophic status, long life span and high site fidelity. Valuable lessons gained from the more highly exposed wildlife species, such as dolphins, necessitates

us to better integrate human and ecological research to all consumers to assess health risks of persistent contaminants. Because the risk posed by exposure to these compounds through intake of fish species is a matter of concern to both humans and dolphins, continued monitoring and more complete evaluation of potential sources will help to provide further understanding of PFAS distribution.

3.4.2. Health aspects

Studies, both laboratory and epidemiology, support the potential for negative health outcomes from PFAS exposures. Since the levels of PFAS in fish are relatively high among food items, the intake of contaminated fish may be a significant source of these contaminants (Domingo and Nadal, 2017; Falandysz et al., 2006; Berger et al., 2009). Several studies have shown that PFAS levels in human blood positively correlated with consumption of fish (Weihe et al., 2009; Rylander et al., 2009). Although fish need to be considered as an important ongoing source of contaminant exposure, fish is also a good source of protein and omega-3 fatty acids and consumption also has positive health benefits such as reduced risk of cardiovascular disease and mortality (He et al., 2004).

4. Conclusions

Our study found PFOS concentrations of certain fish species and locations consumed by humans and wildlife (dolphins) exceed human health and wildlife values. All fish show PFAS contamination with lowest levels occurring in mullet and highest levels in all other species (croaker, spot, red drum, seatrout, and flounder). While the risk/benefit assessment is complicated, consumption of several species of fish including from the Charleston Harbor and its tributaries may pose risks as PFAS (especially PFOS) were identified as potential chemicals of concern. The PFOS concentrations in fillets exceeded human screening values for cancer risk in certain species and locations. The detected residues of PFOS found in fish from Charleston estuarine waters may be a potential risk for the health of consumers with elevated fish consumption. Thus, there is a need to conduct more studies on fish in areas that are fished by recreational and subsistence consumers, screening level risk assessments with further studies on contaminant sources and mitigation measures for a cleaner environment. In the meantime, consumption advisories should be considered as a prudent public health measure.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2019.01.021.

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