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Per- and polyfluoroalkyl substances (PFAS) in aquaculture feeds and potential dietary exposure to and from aquaculture fish

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ABSTRACT

The "forever chemicals" per- and polyfluoroalkyl substances (PFAS) have been measured in wild caught fish, but few studies have examined PFAS in aquaculture. This study aims to quantify PFAS in commercial aquaculture feeds, quantify PFAS in fillets and livers of aquaculture fish fed commercial diets, and estimate human dietary exposure of PFAS through consumption of aquaculture fish. Thirteen commercial feeds were analyzed for 29 PFAS. Fifteen PFAS were detected with perfluorooctanesulfonic acid (PFOS) detected in all feeds. A 63-day feeding trial on juvenile red drum (*Sciaenops ocellatus*) fed two different feeds at three ration levels was conducted. Only PFOS was detected in the fillets and livers, and fish given the higher PFOS diet exhibited a higher percentage of detection in their fillets (70.8 %; mean = 0.09 ng/g) compared to fish given the lower PFOS diet (0 %). There was a significant difference in size between the feed groups (p-value < 0.0001), suggesting a possible growth dilution effect. PFOS did not differ among rations, and levels in the fillets did not exceed EFSA guidelines. This study provides baseline data on dietary contribution of PFAS to and from aquaculture fish and allows for informed safe consumption recommendations to safeguard human health.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of anthropogenic chemicals that have been utilized in a variety of industrial and consumer products since the 1950s due to their thermal stability and resistance to water and oil (Buck et al., 2011; Kwiatkowski et al., 2020). The strong C–F bonds give PFAS their unique chemical properties, and they do not readily break down under various environmental conditions, resulting in widespread contamination of water, soil, wildlife, and humans (Henry et al., 2018).

Elevated PFAS exposure has linked kidney and testicular cancer, liver disease, decreased fertility, thyroid problems, hormone disruption, immunotoxicity, and developmental toxicity (Barry et al., 2013; DeWitt et al., 2012; Lopez-Espinosa et al., 2012; Zhang et al., 2015). Dietary exposure guidelines have not yet been established in the U.S. but the European Food Safety Authority (EFSA) has set a tolerable weekly intake (TWI) for the sum of four PFAS (perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexanesulfonic acid (PFHxS)) from food at 4.40 ng/kg body weight (BW)/week.

Human exposure to PFAS through seafood is an exposure route of concern. Studies have demonstrated associations between fish and shellfish consumption and elevated serum PFAS concentrations in humans (Christensen et al., 2017; Sunderland et al., 2019). Many PFAS are bioaccumulative in nature (Conder et al., 2008), and edible fish species of higher trophic levels, such as tuna, salmon, and cod, face an increased risk of PFAS accumulation due to the biomagnification of PFAS up the food chain (Miranda et al., 2023; Xu et al., 2014). Therefore, monitoring PFAS levels in seafood and implementing measures to mitigate contamination are crucial to safeguarding public health.

Despite studies suggesting that wild-caught seafood is a major source of PFAS exposure, little is known about PFAS in aquacultured fish or possible exposure routes to these fish. One study found perfluoroalkyl acids (PFAAs), a subgroup of PFAS, were present in wild freshwater fish in Finland, but were not detected in farmed fish (Koponen et al., 2015). However, another study found at least ten PFAS in the blood and tissue

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of farmed freshwater fishes, with PFOS accounting for more than half of the total concentrations of PFAS in all blood and tissue samples (Shi et al., 2012). Therefore, it is evident that further research on PFAS in aquaculture, as well as possible exposure routes, is warranted to better understand the risk farmed fish may pose to humans who consume them.

One potential source of contamination in aquaculture systems is commercial feed, which commonly contains a high protein ingredient called fish meal. Fish meal is derived from the processing wastes of wild-caught marine fish that have been harvested for human consumption (Rust et al., 2011). Because PFAS bind preferentially to proteins rather than lipids (Alesio et al., 2022; Woodcroft et al., 2010), the bioaccumulation of PFAS in the protein of fish meal potentially leads to their incorporation into aquaculture feeds. However, thermal processing can potentially reduce PFAS concentrations in seafood (Vendl et al., 2022). Therefore, gaining a better idea of how much PFAS is present in aquaculture diets and fish is important for establishing safe consumption guidelines.

Because the amount of food given to fish in aquaculture settings can be controlled, there is a potential to limit the amount of dietary PFAS through rationing. Proper feeding in aquaculture is crucial in order to maximize growth efficiency and profitability while minimizing overfeeding and waste. Two common feeding strategies in aquaculture include feeding to apparent satiation and feeding based on a percent body weight (BW) per day. Generally, daily rations may range anywhere from 0.5 % BW/day to 10 % BW/day, depending on species and life stage (Patillo, 2014). Due to the range of ration sizes utilized in aquaculture, the amount of PFAS exposure to aquaculture fish through the diet may vary, and to our knowledge, there have been no studies done to evaluate the effects of dietary ration sizes on PFAS bioaccumulation.

Ultimately, seafood is a nutritious food source with much of the world supply coming from aquaculture thereby warranting the investigation of human PFAS exposure through consumption of aquacultured fish.

The objectives of this study are threefold. (1) Quantify PFAS in commercial aquaculture feeds of varying percent protein, protein sources, and manufacturers. (2) Quantify PFAS in the fillet and liver, of aquaculture fish raised on two feeds with different contamination levels of PFAS and three different ration sizes to assess how feed influences concentrations of PFAS in the edible portion as well as a bioaccumulative organ. (3) Estimate potential dietary exposure of PFAS to humans through consumption of aquaculture fish fillets using the fillet results of objective two.

2. Materials and methods

2.1. Standards and chemicals

A PFAS standard stock solution containing 29 PFAS (Table 1) at a concentration of 1.26 ng/g in methanol purchased from Wellington Laboratories (Product Number: PFAC-30PAR, Guelph, Ontario). Eight calibration solutions (79.9 ng/g to 0.52 ng/g) were created by gravimetrically diluting the stock into three working solutions in methanol. A concentrated stock of 19 stable isotopically labeled PFAS purchased from Wellington Laboratories (Product Number: MPFAC-24ES) was used to create an internal standard (IS) solution at 2.2 ng/g.

Eight blanks using nanopure water at 18.2 Mohm and eight aliquots from one jar of NIST Standard Reference Material (SRM) 1947a Lake Superior Fish Tissue were analyzed and used as a control material to assess the precision of the method.

2.2. Feed analysis

2.2.1. Feed Sample Selection

Thirteen commercial aquaculture feeds from five manufacturers (MFR) were selected and analyzed for PFAS based on % crude protein (CP) and ingredient composition. Ingredient composition and CP were obtained from manufacturer-provided feed labels and were not independently verified through analytical testing. Feeds specifically

Table 1
PFAS analytes, abbreviations, internal standards, chain-length categorization, and diagnostic ions used in the determination of the target analytes.

Analyte	Abbreviation	Internal Standard	Chain Length	Precursor (m/z)	Product 1 (m/z)	Product 2 (m/z)
Perfluoro-*n*-butanoic acid	PFBA	13C4-PFBA	Short	213	169	N/A
Perfluoro-*n*-pentanoic acid	PFPeA	13C5-PFPeA	Short	263	219	119
Perfluoro-*n*-hexanoic acid	PFHxA	13C5-PFHxA	Short	313	269	119
Perfluoro-*n*-heptanoic acid	PFHpA	13C4-PFHpA	Short	363	319	169
Perfluoro-*n*-octanoic acid	PFOA	13C8-PFOA	Long	413	369	169
Perfluoro-*n*-nonanoic acid	PFNA	13C9-PFNA	Long	463	419	219
Perfluoro-*n*-decanoic acid	PFDA	13C6-PFDA	Long	513	469	219
Perfluoro-*n*-undecanoic acid	PFUnA	13C7-PFUnA	Long	563	519	269
Perfluoro-*n*-dodecanoic acid	PFDoA	13C2-PFDoA	Long	613	569	269
Perfluoro-*n*-tridecanoic acid	PFTriA	13C2-PFTA	Long	663	619	269
Perfluoro-*n*-tetradecanoic acid	PFTA	13C2-PFTA	Long	713	669	369
Perfluoro-1-hexanesulfonamide	PFHxSA	13C3-PFHxS	Long	398	78	169
Perfluoro-1-octanesulfonamide	PFOSA	13C8-PFOSA	Long	498	78	169
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	HFPO-DA	13C8-PFOS	Short	329	285	169
N-methylperfluoro-1-octanesulfonamidoacetic acid	NMeFOSAA	d3-NMeFOSAA	Long	570	419	512
N-ethylperfluoro-1-octanesulfonamidoacetic acid	NEtFOSAA	d5-NEtFOSAA	Long	584	419	526
Perfluoro butanesulfonate	PFBS	13C3-PFBS	Short	299	80	99
Perfluoro pentanesulfonate	PFPeS	13C3-PFOS	Short	349	80	99
Perfluoro hexanesulfonate	PFHxS	13C3-PFHxS	Long	399	80	99
Perfluoro heptanesulfonate	PFHpS	13C3-PFOA	Long	449	80	99
Perfluoro octanesulfonate	PFOS	13C8-PFOS	Long	499	80	99
Perfluoro nonanesulfonate	PFNS	13C8-PFOS	Long	549	80	99
Perfluoro decanesulfonate	PFDS	13C8-PFOS	Long	599	80	99
1H,1H,2H,2H-perfluoro-1-hexanesulfonate	4:2FTS	13C2-4:2FTS	Long	327	307	81
1H,1H,2H,2H,-perfluoro-1-octanesulfonate	6:2FTS	13C2-6:2FTS	Long	427	407	81
1H,1H,2H,2H,-perfluoro-1-decanesulfonate	8:2FTS	13C2-8:2FTS	Long	527	507	81
Dodecafluoro-3H-4,8-dioxanonatoate	NaDONA	13C8-PFOS	Long	377	251	N/A
9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl-PF3ONS	13C3-PFOS	Long	531	351	353
11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUnS	13C8-PFOS	Long	631	451	N/A

designed for red drum, salmon, trout, tilapia, and catfish were selected due to those species' high rates of consumption by people. Feeds containing various protein ingredients were chosen, including both general marine feeds utilizing animal/marine (A/M)-based ingredients such as fish meal, feather meal, blood meal, and plant-based feeds using ingredients such as soybean meal and corn gluten meal. All feeds contained both A/M-based and plant-based ingredients, so they were categorized based on the dominant group, determined by both the placement of ingredients on the ingredients list as well as the number of A/M or plant-based ingredients. Furthermore, a range of % CP within the feeds was selected. Each feed was assessed in triplicate.

2.2.2. Feed homogenization

All feeds were cryogenically homogenized using a precleaned CryoMill (Retsch, Haan, Germany) to ensure sample uniformity, and all components were precleaned with methanol. Approximately 9 g of feed was placed in a pre-cooled jar with grinding balls and capped closed. The jar was then placed into the grinder and allowed to pre-cool to a temperature of −196 °C for 1 min before being homogenized for 1 min at 250 Hz. Homogenate was then placed into a pre-cooled 50 mL polypropylene (PP) centrifuge tube. This process was repeated until approximately 20 g were obtained for one feed sample.

2.2.3. Feed Sample preparation

Samples were prepared for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis according to the methods developed by the FDA for determining PFAS in processed food (Genualdi et al., 2022). Briefly, a Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method was used for extracting PFAS from 5 g (gravimetrically tracked) of homogenized feed amended with 600 μL of 2.2 ng/g isotopically labeled standard solution, 5 mL of nanopure water (18.2 Mohm), 10 mL of acetonitrile, and 150 μL of formic acid. The centrifuge tube was then shaken vigorously for 1 min, after which a QuEChERS salt packet containing 6000 mg $MgSO_4$ and 1500 mg NaCl (UCT, Bristol, PA) was added. The tube was then centrifuged and the supernatant transferred to a 15 mL PP centrifuge tube pre-filled with dSPE sorbent (900 mg MgSO4, 300 mg primary secondary amine, 150 mg graphitized carbon black) (UCT, Bristol, PA, Product Number: ECMPSCB15CT) for lipid removal and again centrifuged at room temperature. A 0.2 μm nylon syringe filter on a barrel syringe was used to filter the supernatant into a clean 15 mL PP centrifuge tube. One mL of filtrate was then added to an autosampler vial for LC-MS/MS analysis.

2.3. Fish tissue analysis

2.3.1. Fish rearing

Juvenile red drum (*Sciaenops ocellatus*) were obtained through volitional spawning at the Marine Resources Research Institute (MRRI) in Charleston. Red drum are a popular recreational sport fish found on the coast of the southeast U.S. and the Gulf of Mexico, and farming production of red drum has been increasing due to their exceptional growth in aquaculture settings and adaptability to a wide range of salinities. Furthermore, their nature as generalist feeders makes them an ideal model species for feeding studies, as they can consume a diverse range of commercial feeds. Once hatched, the larval red drum were transported to Waddell Mariculture Center in Bluffton, SC, and stocked in ponds until they reached an approximate length of 30 mm to 40 mm. The fish were then transferred back to MRRI, stocked in a recirculating aquaculture system, and fed daily to apparent satiation with a commercial feed, from this point forward referred to as the "initial feed". Once the fish reached an average weight of 40 g, they were transferred to Hollings Marine Laboratory in Charleston, SC and stocked in a twenty-four-tank recirculating aquaculture system equipped with biological and mechanical filtration, UV sterilization, a protein skimmer, and temperature control. Two feeds, Feed A and Feed B, were selected for the feeding trial based on the PFAS results obtained from Section 2.2 and their suitability for juvenile red drum in terms of protein and lipid content.

2.3.2. Background PFAS assessment

Prior to the feeding trial, background concentrations of PFAS in the water as well as PFAS that could leach from the feeds into the water was assessed. Because fish can uptake PFAS from water through their gills, PFAS from the water could obscure our understanding of PFAS accumulation in fish due to dietary exposure. To assess potential PFAS contamination originating from the tank system or water, four 4-liter water samples were collected after the water had been recirculating throughout the system for several weeks, prior to stocking the fish in the recirculating aquaculture system at Hollings Marine Laboratory. To achieve a proportional representation of the water-to-pellets ratio within the tanks, approximately 0.2 g of each feed was separately added to two of the 4 L of water and sonicated (Fisher Scientific, Model FS220) at room temperature for 20 min. Pellets were then removed using a precleaned laboratory spatula, and water was divided into four 250 mL PP bottles. Internal standard solution (mass known) was added to each bottle. Solid phase extraction (SPE) was then conducted on the samples according to EPA Method 533: Determination of per- and polyfluoroalkyl substances in drinking water (Rosenblum and Wendelken, 2019). Briefly, SPE cartridges were rinsed with methanol and nanopure water. Water samples were then added to the cartridges, and bottles rinsed with 10 mM ammonium acetate in water and drawn through the cartridge. Methanol was added to the sample bottle and drawn through the cartridges to dryness. Sample collection tubes were placed under each SPE cartridge and sample bottles rinsed with 10 mL of methanol with 2 % ammonium hydroxide and drawn through the cartridge. Samples were then dried under nitrogen to approximately one mL and analyzed using LC-MS/MS. No PFAS were detected in any of the water samples, indicating there was no existing PFAS in the water used and that the feeds did not leach PFAS into the water.

2.3.3. Feeding trial

Two weeks before the feeding trial, fish were separated into densities of 25 fish per tank and continued to be fed to apparent satiation on the initial feed. One day before the feeding trial, one fish per tank was randomly selected and sacrificed in a lethal solution of diluted tricaine mesylate according to South Carolina Department of Natural Resources protocol, and fillets collected to obtain baseline PFAS concentrations. Whole body mass (WBM), standard length, and fillet mass were recorded, and fillets were stored in plastic bags and stored at −80 °C until analysis. The total BW of each tank with the remaining 24 fish was recorded.

Both feeds, as well as the initial feed, were analyzed for PFAS prior to the experiment according to the methods described in Section 2.2.4. Enough feed was purchased to provide the same respective lot throughout the feeding trial. Each of the 24 tanks were randomly assigned to one of two groups, Group A or Group B, and fed its corresponding feed. The two feed groups were further divided into three distinct ration levels (high, medium and low), yielding a total of six unique groups with four replicates per group. From Days 1 to 33 of the trial, the daily rations for each feed were 2 % BW/day (low), 4 % BW/day (medium), and 6 % BW/day (high). Due to consistent observations of leftover feed in the medium and high groups, on Day 34, four fish from each tank were randomly selected and sacrificed, and fillets were collected for PFAS analysis. Daily rations were then lowered to 1 % BW/day, 2 % BW/day, and 3 % BW/day to ensure better feeding efficiency. For the remainder of the trial, fish were given their respective feed and new ration, and leftover feed was minimal.

Daily rations were split into two feedings each day, and fish were allowed to feed for approximately 15 min, after which any leftover feed was flushed out of the tank. Temperature of the water was recorded daily, while dissolved oxygen, salinity, and pH were measured three times per week using a YSI probe, and ammonia and nitrite were measured once per week using a spectrophotometer (Hach Company,

Model DR/2500) (Table S1). Every two weeks, fish weights were recorded per tank, and rations were adjusted accordingly. At the conclusion of the trial, Day 63, four more fish from each tank were sacrificed and fillet and liver tissues collected and stored in plastic bags and frozen at −80 °C until analysis.

2.3.4. Tissue Sample preparation

Fillet and liver samples were prepared for LC-MS/MS analysis following Method 2 described by Reiner et al. (2012). Briefly, samples were thawed, the outer tissue removed, and approximately 1 g of tissue was homogenized and weighed into polypropylene tubes along with calibrants, SRMs, and blanks. After addition of internal standards, samples were extracted with methanol, cleaned using Supelclean ENVI-Carb SPE cartridges, evaporated under nitrogen, and reconstituted for LC-MS/MS analysis.

2.4. LC-MS/MS analysis

All feed, muscle, and liver samples were analyzed using an Agilent Infinity II liquid chromatography instrument coupled to a SCIEX Triple Quad 5500+ LC-MS/MS. A Zorbax Diol (4.6 mm ID, 12.5 mm, 6 μM particle size) in series with an Agilent InfinityLab Poroshell 120 EC-C18 column (4.6 mm ID, 100 mm, 2.7 μM particle size). Each run involved a ramping LC solvent gradient with methanol and deionized water both containing 10 mmol/L ammonium acetate (Table S2). Two multiple reaction monitoring (MRM) transitions, if available, were employed for each PFAS, one for quantitation and the other for confirmation of the PFAS (Table 1). Manual integrations were performed using ABSciex Analyst Software 1.7. Mass fractions of 29 PFAS in the samples were calculated using Microsoft Excel. Limit of detection (LOD) was defined as either the concentration of the lowest calibrant or the average of the blanks plus three times the standard deviation in the blanks, whichever was larger.

2.5. Data analysis

All statistical analysis was performed using RStudio version 4.2.1 (R Core Team, 2022). Data were tested for normality using Shapiro-Wilks test and for homogeneity of variances using Levene's test. If data was found to be non-normal, the appropriate nonparametric tests were selected.

2.5.1. Feed analysis

Statistical analysis was performed only on PFOS, as it was the only compound present in ≥75 % of feeds tested. Sample measurements below the LOD were assigned a value of one-half the LOD (Table S3). Mean PFAS concentrations of the three replicates for each feed were calculated. Data were found to be non-normal ($p = 0.04$). Therefore, a Mann-Whitney *U* test was run to compare PFAS between the two protein source groups and Kruskal-Wallis tests were performed to determine if MFR had an effect on PFAS concentration and if protein percentage in the feeds influences PFAS concentrations. MFRs B and E were excluded from MFR analysis due to low sample size.

2.5.2. Fillet and liver analysis

Of the 29 compounds measured, only PFOS was detected above the LOD in the fillets. All fillet samples from Day 0 had detectable levels of PFOS. However, by Day 34, 29 % of samples in Group A and 100 % of samples in Group B were below the LOD, and at the conclusion of the trial, approximately 42 % of samples within Group A were below the LOD and all samples within Group B remained below the LOD. Thus, statistical tests were conducted according to Helsel's methods of survival analyses for censored data (Helsel, 2012). The mean PFOS concentration within each tank was calculated, and samples falling below the LOD were assigned the LOD as their representative value.

Peto-Peto tests were first conducted to compare the PFOS distributions in the fillets and livers between the rations within the feed groups. A Peto-Peto test is a modified log-rank test specifically designed to handle datasets containing censored data, making it a suitable choice when analyzing data in which many samples are below the LOD. However, no relationship was observed between ration and fillet or liver PFOS concentrations. Additionally, PFOS was not detected in any of the fillets of Group B and were therefore omitted from analysis, and PFOS concentrations were not significantly different among the ration groups of Group A. Due to the lack of significant relationship between PFOS concentration and ration in this study, the different ration groups were combined for the remainder of the analyses.

Due to large differences in size of the fish between the feed groups over the course of the trial, an Akritas-Theil-Sen (ATS) regression was conducted to estimate the slope of the relationship between size of the fish (mean WBM) and PFOS concentration in the fillets of both feed groups. Additionally, Kruskal-Wallis tests were conducted to determine significant differences in WBM between the groups. A Cox proportional hazards model was then employed to determine differences in the probability of detection of PFOS in the fillets between the feed groups, and WBM was included as a covariate in the model. Next, Peto-Peto tests were conducted to compare the distributions of PFOS in the fillets between the initial group, and Groups A and B both on Day 34 and Day 63.

Livers were only analyzed at Day 63. Of the 29 compounds measured, only PFOS was detected above the LOD in the livers. Peto-Peto tests were used to compare PFOS distributions across Group A fillets versus livers and Group A livers versus Group B livers. Because PFOS was not detected in any fillets within Group B on Day 63, no statistical analyses were conducted to compare Group B fillets versus livers. ATS regression was conducted to estimate the slope of the relationship between WBM and PFOS in the livers, and a Cox proportional hazards model was conducted to determine differences in the probability of detection of PFOS in the livers between the feed groups.

2.6. Dietary exposure estimates

Dietary exposure estimates were calculated to assess potential weekly dietary exposure to PFOS per kg of body weight (BW). Weekly dietary exposure (WDE) (ng/week) was first calculated using Equation (1). The USDA's recommended weekly seafood intake of eight oz (227 g)/week was used for calculations (U.S. Department of Agriculture & U.S. Department of Health and Human Services, 2020). Next, WDE per kg BW was calculated using Equation (2). A BW of 80.0 kg was used to calculate adult exposure, and 15.0 kg BW was used to calculate child exposure.

$$WDE\left(\frac{ng}{week}\right) = PFOS\ concentration\ (ng\ /\ g) \times \frac{227\ g}{week} \qquad \text{(Equation 1)}$$

$$WDE\ per\ kg\ BW = \frac{WDE\left(\frac{ng}{week}\right)}{kg\ BW} \qquad \text{(Equation 2)}$$

Median, upper bound (maximum concentration) and lower bound (minimum concentration) scenarios were calculated. These results were compared to the TWI threshold of 4.40 ng/kg BW/week set by the EFSA to determine if the fillets of fish given commercial aquaculture feeds surpass the weekly dietary recommendations.

3. Results

3.1. PFAS in commercial aquaculture feeds

Fifteen of the 29 PFAS investigated were measured at detectable levels in at least one of the feeds (Fig. 1). The four most frequently detected compounds, PFOS, PFNA, PFDA, and PFUnA, are all long-chain compounds. Other long-chain compounds detected include PFTriA, PFOA, PFOSA, PFNS, PFDoA, PFHxS, and PFTA. There were also four

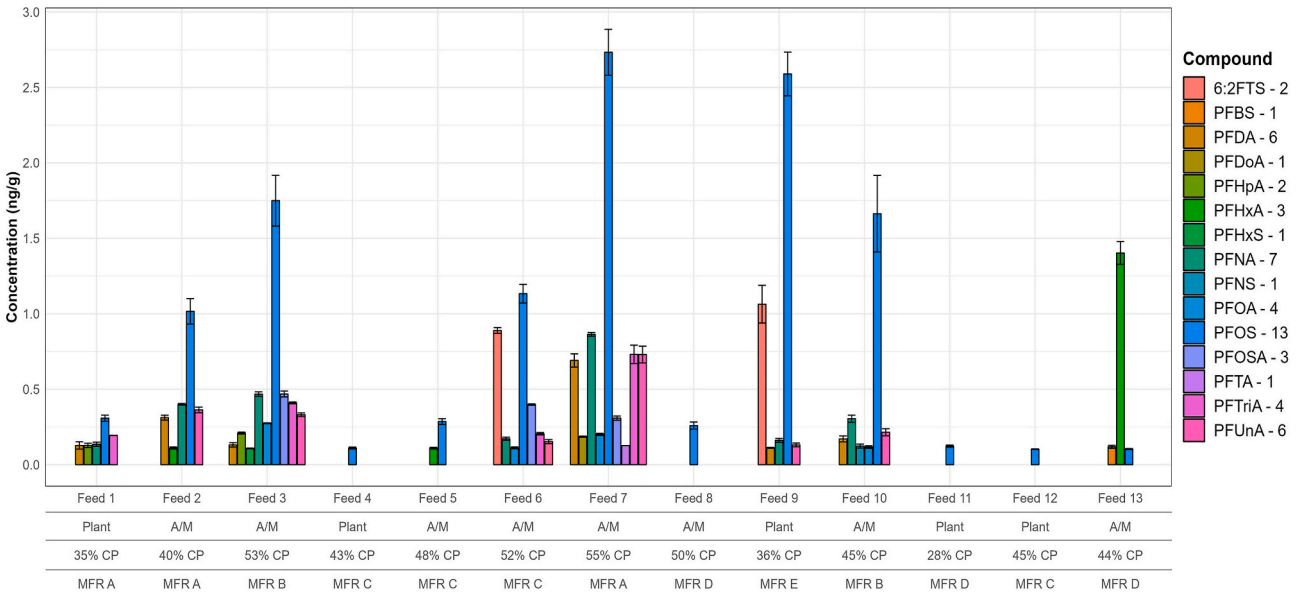

Fig. 1. Individual PFAS concentrations (ng/g) observed in each feed tested. Feeds are labeled by number, category (animal/marine [A/M] or plant), percent crude protein (% CP), and manufacturer (MFR). Numbers next to each compound indicate the total number of feeds from which that compound was detected. Error bars represent the standard error of the mean.

short-chain compounds detected: PFHxA, PFHpA, 6:2 FTS, and PFBS. PFOS was the only compound detected in all thirteen feeds, with a maximum concentration of (2.73 ± 0.26) ng/g in Feed 7 and a minimum concentration of (0.103 ± 0.002) ng/g in Feed 12 (Fig. 1). Feed 3 and Feed 7 contained the most compounds, with nine total PFAS detected in each; however, specific compounds varied between the two. Feeds 4, 8, 11, and 12 all contained just one compound, PFOS (Fig. 1).

Among the A/M-based feeds, only one feed contained solely PFOS, Feed 8. The remaining feeds in this category exhibited a range of two to nine individual compounds. The highest concentration measured in the A/M-based feeds was PFOS at 2.73 ng/g in Feed 7. Three of the five plant-based feeds, Feeds 4, 11, and 12, only contained one compound, PFOS, and the remaining two plant-based feeds, Feed 1 and 9, each contained five distinct compounds (Fig. 1). The highest concentration measured in the plant-based feeds was PFOS at 2.59 ng/g in Feed 9. Overall, there were no significant differences in PFOS concentration between A/M-based feeds and plant-based feeds (Mann-Whitney U, $p = 0.5$).

Generally, MFRs A and B exhibited the highest number of detections among all analyzed feeds, with 11 individual PFAS across the three feeds from MFR A, and 10 individual PFAS among the two feeds from MFR B. MFR D exhibited the lowest PFAS detection count, identifying only three compounds—PFOS, PFHxA, and PFBS—across the two feeds analyzed. Overall, there were no significant differences in PFOS concentration among the manufacturers (Kruskal Wallis, $p = 0.2$).

There was no significant correlation between PFOS concentration and % CP (Kruskal-Wallis, $p > 0.6$). The four feeds that only contained PFOS had a wide range of 28 % to 50 % CP. Therefore, % CP is not the main indicator of whether a feed will contain higher levels of PFAS.

3.2. PFAS in fillet and liver tissue

A total of 204 fillets were sampled and analyzed: 12 from the initial group, 96 from Group A, and 96 from Group B. PFOS was the only compound detected in the fillets at any given time point, with the maximum PFOS concentration detected being 0.193 ng/g in the initial

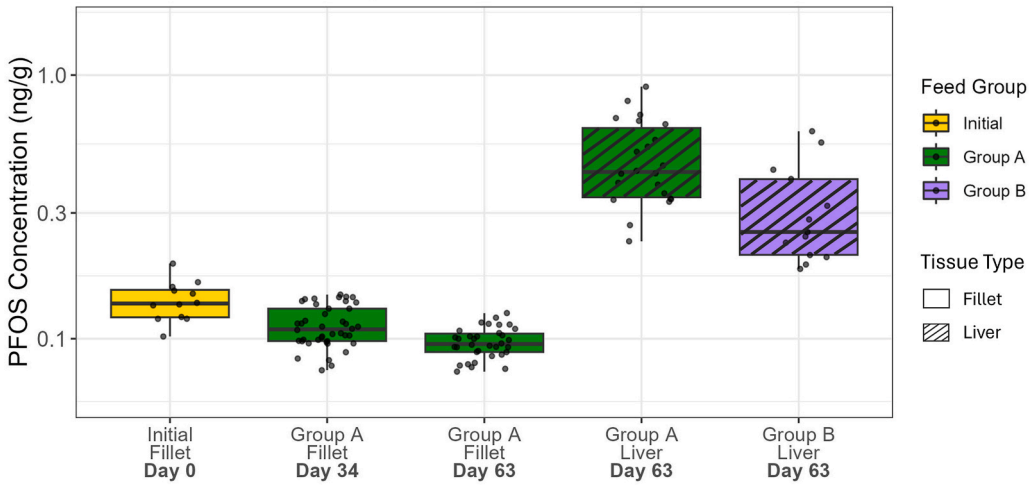

Fig. 2. PFOS concentrations (ng/g) found in Initial fillets, Group A fillets and livers, and Group B livers. The boxes represent the interquartile range, the horizontal line in each box represents the median PFOS concentration, and the whiskers represent data outside the interquartile range.

group on Day 0. According to the Peto-Peto tests, median PFOS in the fillets of the initial group from Day 0 was 0.135 ng/g (Fig. 2). On Day 34, median PFOS in fillets of Group A was 0.111 ng/g, and all fillet samples within Group B were below the LOD; therefore, no median PFOS was calculated. By Day 63, median PFOS in Group A was 0.0920 ng/g and PFOS in Group B was still not detectable. According to the Cox proportional hazards model, Group A had a significant effect on the probability of detection of PFOS in the fillets compared to Group B ($p = 0.0004$; Fig. 3). In summary, the initial group had the highest PFOS fillet detections, Group A had approximately 58 % detection above the LOD and lower concentrations than the initial group, and Group B had 0 % detection above the LOD (Fig. 3). There were significant differences in WBM between the groups (p-value < 0.0001), with Group B being significantly larger than Group A throughout the trial (Fig. 4a). Furthermore, ATS regression found that the slopes of the line for both Groups A and B were significantly different from zero, indicating a strong relationship between size and PFOS ($p = 0.03$; $p = 0.0003$, respectively; Fig. 4b).

A total of 95 livers were sampled and analyzed: 47 from Group A, and 48 from Group B. PFOS was the only compound detected in the livers with the maximum PFOS concentration detected being 0.904 ng/g from Group A. According to Peto-Peto tests, PFOS was generally higher in the livers than in the fillets of Group A ($p = 0.007$), with a median PFOS concentration of 0.525 ng/g in the livers, compared to a median PFOS concentration of 0.092 ng/g in the fillets (Fig. 2). Additionally, PFOS concentrations in Group A livers were higher than in Group B livers ($p < 0.0001$), with a median PFOS concentration of 0.243 ng/g in Group B livers. ATS regression showed no significant relationship between body size and PFOS concentrations in livers for either group (Group A: $p = 0.7$; Group B: $p = 0.8$). This lack of significance is likely attributable to sampling on a single day, as opposed to throughout the trial, which limited both sample size and the range of body masses. Lastly, according to Cox proportional hazards model, similarly to fillets, Group A had a significant effect on the probability of detection of PFOS in the livers compared to Group B ($p = 0.0006$; Fig. 3).

3.3. Dietary exposure estimates

Dietary exposure estimates were calculated for PFOS only, as it was the only compound detected above the LOD in the fillets, which is considered the edible portion of the fish for this study, at any time point. Weekly dietary exposure (WDE) estimates for median, upper bound, and lower bound scenarios are presented in Table 2. For adults, WDE/kg BW ranged from 0.255 ng/kg BW/week to 0.548 ng/kg BW/week. For children, estimates ranged from 1.36 ng/kg BW/week to 2.92 ng/kg BW/week.

4. Discussion

4.1. PFAS in commercial aquaculture feeds

PFAS were detected in all commercial aquaculture feeds in varying quantities and concentrations, with PFOS consistently the dominant compound. Four feeds contained solely PFOS, consistent with a previous report by Rushing et al. (2023), which found that PFOS was the sole compound detected in two brands of fish flakes tested. Furthermore, in this study, PFOS was detected at the highest concentrations across all the measured compounds, comparable to Cao et al. (2022), which found that PFOS was the highest detected compound in a study that analyzed PFAS in laboratory fish diets. Thus, this study underscores the prevalence of PFOS in a variety of fish feeds.

When comparing the two categories of feeds and their PFOS concentrations, there was no significant difference found between A/M-based feeds and plant-based feeds. This is somewhat surprising since PFAS concentrations are generally higher in animal and fish tissues, which are utilized in the production of A/M-based feeds, compared to plants like soy or corn (Domingo et al., 2012). Ingredients that are commonly used in both A/M-based feeds and plant-based feeds, such as fish meal, feather meal, blood meal, and soybean meal, could play potential roles in contributing to PFAS contamination within the feeds. Therefore, it should be noted that source ingredients were not tested in this study and there is potential for PFAS contamination to come from any and multiple ingredients. For example, Suominen et al. (2011) found that PFAS in fish meal samples ranged from 0.9 ng/g to 20 ng/g, and another study found that PFAAs were detected in all sixteen fish meal samples tested, with a mean concentration of 18.2 ng/g (Li et al., 2019). The majority of fish meal is produced from the processing wastes of whitefish (Rust et al., 2011), and the concentrations of PFAS detected in wild-caught whitefish have been found to vary depending on both the species and geographical location (Ruffle et al., 2020; Zafeiraki et al.,

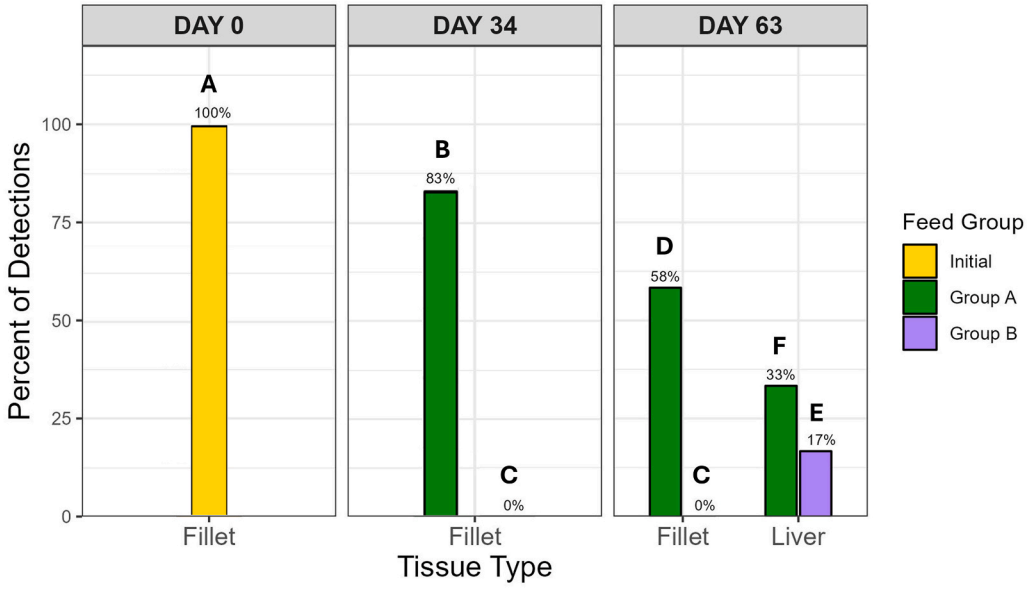

Fig. 3. The percentage of detection of PFOS in fillets and livers within each group on Days 0, 34, and 63, found to be significantly different between groups (Cox proportional hazards model; $p < 0.05$).

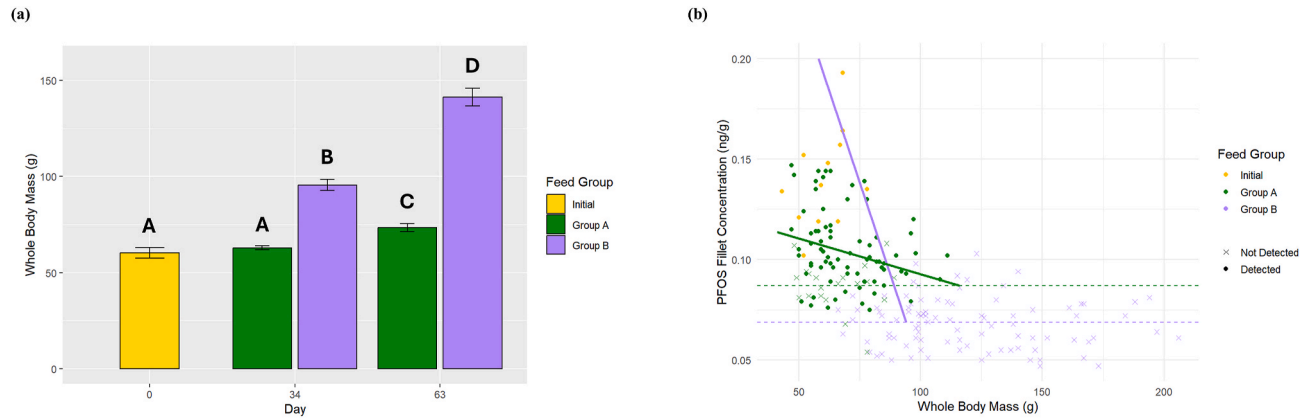

Fig. 4. (a) Mean whole body mass (g) of fish in the initial group on Day 0 and Groups A and B on Days 34 and 63. Error bars represent the standard error of the mean. Letters above each bar indicate significance between groups. (b) ATS regression of PFOS in fillets vs. whole body mass. For non-detect samples, the limit of detection (LOD) value was plotted. Horizontal dashed lines indicate the mean LOD value of undetected samples within each group. The slopes of the lines of both Group A and Group B were significantly different from zero, according to Akritas-Theil-Sen regression ($p = 0.03$; $p = 0.0003$, respectively).

Table 2
Estimated weekly dietary exposure (WDE) and WDE per kg BW for adults and children based on PFOS concentrations detected in fillet samples from the present study, regardless of time point. Median, upper bound, and lower bound scenarios were calculated. WDE/kg BW was compared to the EFSA's tolerable weekly intake of 4.40 ng/week/kg BW. No estimates surpassed this threshold.

Dietary Exposure Estimates			
	Median	Upper Bound Scenario	Lower Bound Scenario
WDE (ng/week)	26.6	43.8	20.4
WDE/kg BW Adult (ng/week/kg BW)	0.332	0.548	0.255
WDE/kg BW Child (ng/week/kg BW)	1.77	2.92	1.36

2019). Therefore, it is expected that PFAS in fish meal will vary depending on both the species used and the geographic location of where those fish were caught. Feather meal is widely used in commercial aquaculture feeds, and Li et al. (2019) found that concentrations of PFAAs in feather meal ranged from 1.13 ng/g to 9.89 ng/g, and PFOS was the dominant compound with a mean concentration of 1.34 ng/g, which is comparable to the levels of PFOS found in the feeds of this study. Blood meal has been found to contain PFAS in concentrations between 4.26 ng/g to 37.1 ng/g dry weight (Li et al., 2019), and PFOS and PFHxS have been found in livestock cattle serum (Drew et al., 2021; Lupton et al., 2022). Therefore, the use of blood from livestock in the production of blood meal for fish feeds introduces a potential source of PFAS in feeds. In plant-based feeds, a common protein source utilized is soybean meal. As the aquaculture industry is expected to experience substantial growth in the next decade, there is a trend towards adopting more plant-based protein sources in aquaculture feeds, and soybean meal has been widely researched as a potential alternative (Macusi et al., 2023). PFAS have been detected in soybean meal in the range of 1.14 ng/g to 2.13 ng/g, which closely matches the sums of PFAS in the plant-based feeds of this study (Li et al., 2019). Furthermore, the pods of soybean plants found near a fluorochemical industrial park were found to contain concentrations up to 2861 ng/g (Liu et al., 2019). Therefore, similarly to how PFAS in fish meal can vary based on the source of fish, the levels of PFAS in soybean meal can be heavily impacted by the initial source of the soybean plants used in production. In this study, PFOS did not appear to correlate with any one particular protein source. Fish meal, feather meal, blood meal, and plant-based protein sources can vary depending on the species, geographic location, or growing methodologies. While comparison of summed measured PFAS relies on the measurement method and the number of PFAS assessed, the results of this study are within the range of others, likely due to the prominence of PFOS in most feeds.

Long-chain PFAS predominated across feeds, consistent with Choi et al. (2023), which found that of 53 PFAS measured in animal feeds, long-chain PFCAs and PFSAs were the most frequently detected class. Long-chain PFAS have stronger C–F bonds and are generally more hydrophobic, and thus tend to bind to other particles, leading to more bioaccumulation (Ahrens and Bundschuh, 2014). In a study looking at PFAS in highly consumed seafood products in the U.S., only long-chain PFAS were detected in samples of pollock, the by-products of which are commonly used in the production of fish meal in the U.S. (Young et al., 2022). Therefore, the prevalence of long-chain PFAS in our feeds aligns with the current evidence that long-chain PFAS are more persistent in both organisms and the environment.

Short-chain compounds were detected less frequently than long-chain compounds in the feeds. Short-chain compounds are highly mobile in water and soil, and they are more commonly found in plants compared to long-chain PFAS (Brendel et al., 2018). They have also been found in higher concentrations than long-chain PFAS in soybean meal (Li et al., 2019). However, our study found that only two of the five plant-based feeds contained just one short-chain compound. Both feeds also contained four other long-chain compounds, while the remaining three plant-based feeds only contained PFOS. Thus, even though short-chain PFAS have been reported to be more abundant in plants than long-chain PFAS, this study emphasizes that they are not as prevalent in feeds as long-chain compounds.

No significant differences in PFOS concentrations among MFRs were found, though this could be due to the small sample size within each MFR. The number of PFAS detected across MFRs varied but were not directly related to MFR. The variability of PFAS concentrations among manufacturers and across products from different manufacturers has been noted in the literature before (Rushing et al., 2023), and was similarly found to be the case in this study.

Individual PFAS detected also varied within MFRs, which can be attributed to the sources obtained for each lot tested. Thus, it is possible that PFAS can vary from batch to batch within the same MFR and within the same feed. This study did not investigate MFR suppliers nor determine the origin of source material for each MFR. Future studies could examine the extent of PFAS variation between batches of the same feed formulation from the same MFR to assess the consistency of PFAS contamination levels and to identify potential sources of variability within the manufacturing process.

As mentioned, PFAS have high binding affinities to proteins, posing a potential risk of elevated concentrations in fish feeds with higher protein

content. The thirteen feeds tested contained a range of % CP from 28 % CP to 53 % CP; however, no significant relationship was detected between PFOS concentration and % CP. This is consistent with a study that examined the correlation between PFAS concentration and protein content (%) in laboratory fish diets and found no correlation (Cao et al., 2022). Furthermore, the number of individual PFAS showed considerable variability across the feeds. Therefore, protein content does not appear to be a predictor of PFAS concentrations in aquaculture feeds.

4.2. PFAS in fillets and liver

This study revealed that PFAS were either not detected or detected in low concentrations in the fillets of juvenile red drum when fed PFAS-contaminated commercial diets over the course of 63 days. PFOS was the only compound detected in any of the fillets despite consuming feeds containing a diversity of PFAS, indicating that most PFAS are not present in detectable concentrations in aquaculturally-raised juvenile red drum. The results of this study are consistent with a previous study that found PFOS in the highest concentrations in farmed fish, with about 90.4 % of all PFAS observations of the study below the LOD (van Leeuwen et al., 2009). Another study determined that PFAS was not quantifiable in farmed whitefish and rainbow trout (Koponen et al., 2015).

Some studies have compared PFAS in wild-caught and farmed fish and demonstrated that aquaculture fish generally have lower PFAS concentrations in the fillets than wild-caught fish (Barbarossa et al., 2016; Zafeiraki et al., 2019). There are a few possible explanations for the differences in PFAS concentrations seen in wild-caught versus aquaculture fish. First, due to the persistence and high mobility of PFAS, oceans, lakes, and estuaries act as sinks for PFAS, so wild fish that depend solely on foraging may be continuously exposed to PFAS from the aquatic environment. Furthermore, the primary uptake route of PFAS in fish is mainly by passive diffusion through the gills; thus, high levels PFAS in environmental waters can lead to increased accumulation in wild fish (Ng and Hungerbühler, 2013). Conversely, aquaculture facilities are often equipped with filtration systems to maintain water quality. Some of these systems include reverse osmosis filtration or activated carbon filtration, which can help reduce or remove environmental contaminants. The water utilized in the present study was taken from Charleston Harbor, settled for a minimum of 72 h, and put through a polishing filter. In the filter, the water passed through gravel, sand, and activated carbon to remove large particulates and chemical pollutants. The water then went through a micron filter followed by UV sterilization. Despite evidence that PFAS are present in the sediments of Charleston Harbor (White et al., 2015), our leaching study revealed undetectable levels of PFAS in the prepared water used. Additionally, it was found that PFAS from the feeds do not leach into the water during the time the feed is in the tank before flushing; thus, the uptake of PFAS through the gills was deemed less of a concern.

Despite consuming a diet containing a variety of PFAS, the fillets from fish provided either the initial feed or Feed A were found to only contain PFOS, and the fillets from fish provided Feed B had no detectable amounts of any PFAS. Numerous studies that have shown that PFOS is often the dominant PFAS in fish tissue of both farmed and wild-caught fish (Koponen et al., 2015; Pan et al., 2014; Ren et al., 2022; Zafeiraki et al., 2019). Despite efforts to phase out production of PFOS, the continued use of PFOS precursors in the manufacturing of various global commercial and industrial products sustains its prevalence and high concentrations in the environment (Z. Wang et al., 2017). This can explain why PFOS was found in the highest levels in the feeds, facilitating its detection in the fillets. The absence of other PFAS in the fillets that were present in the feeds suggests that PFAS from fish feeds may be excreted through the feces, as observed in rainbow trout (Vidal et al., 2019), or they may be accumulating in tissues other than the muscle, such as the kidney or blood.

Similarly, PFOS was the only PFAS detected in the liver and was found to be higher in the livers than in the fillets of the same group (Fig. 2). This is consistent with the literature that has demonstrated that the highest mean PFOS concentrations was found in the blood of farmed freshwater fish, followed by the liver, brain, and lastly, the muscle (Shi et al., 2012). Similarly, in Cara et al. (2022), fish liver showed a higher mean PFOS concentration compared to the muscle. PFAS have been observed to undergo continuous recirculation between the liver and other organs through enterohepatic recirculation, contributing to the distribution and accumulation of PFAS in the serum, kidney, and liver (Lau et al., 2007), so it is possible that the compounds in the feeds are also accumulating in the blood or kidney of the fish, as opposed to the muscle tissue. Because this study focused on aquaculture fish intended for human consumption, these additional parameters were not analyzed. However, given the higher concentrations in the liver and because PFAS exposure in fish has been linked to health effects, such as behavioral abnormalities and altered gene expression (Haimbaugh et al., 2022; Jantzen et al., 2016), investigating PFAS accumulation and clearance in or from other tissues of aquaculture fish could offer valuable insights into the impact of PFAS-contaminated feeds on fish health.

Interestingly, this study revealed that fillet PFOS concentrations declined during the feeding trial period. In the study, the initial fish exhibited higher concentrations of PFOS than those of Group A, despite being given a diet lower in PFOS. Furthermore, Group A fish were significantly smaller than Group B, indicating a slower growth rate that likely contributed to the detectable levels of PFAS in the fillets as opposed to Group B. However, the relationship between WBM and concentration was negative for both groups (Fig. 4b), suggesting that if the trial had gone on longer, concentrations in Group A would have likely continued to decrease as the fish continued to grow. Furthermore, despite the slow growth rate, Group A did still exhibit some growth dilution, as they were larger and had lower concentrations of PFAS than the initial group by the conclusion of the trial. Therefore, despite PFOS having accumulated in the fillets of the initial group, the fillet growth rate is greater than the PFOS accumulation rate, leading to disproportionate accumulation.

Detections of PFOS in the fillets also declined throughout the study. In both the fillets and livers, Group A had significantly higher percentage of detections than Group B (Fig. 3), which might be attributed to the difference in PFOS concentrations within each feed. Notably, Feed A contained a significantly higher concentration of PFOS than Feed B (Fig. 5). Fish in Group A were also significantly smaller than fish in Group B by the conclusion of the trial (Fig. 4a). The ATS regression analysis showed that there was a significant weak correlation between size (WBM) of the fish and PFOS concentration in the fillets (Fig. 4b). This observation could suggest the occurrence of growth dilution. Some studies have observed this occurrence in other species. For example, a negative correlation between PFOS and daily growth rate was found in white-tailed eagle and goshawk nestlings (Bustnes et al., 2013). Moreover, Hoover et al. (2017) found that the bioconcentration factor of some PFAS decreased in tadpoles during exposure. The juvenile fish of this study, as well as the nestlings and tadpoles of the aforementioned studies, are all at similar life stages characterized by growth. This suggests that when organisms are undergoing steady growth during PFAS exposure, it is possible that concentrations will not accumulate, but decrease, in the organism as dilution occurs. Future studies should investigate whether there exists a maximum concentration of exposure beyond which PFAS begin to accumulate in fillet tissues during growth.

Alternatively, differences in growth between Groups A and B could also be attributed to the differences in PFOS concentrations in the feeds. Feed A was found to have nearly three times the concentration of PFOS than Feed B (Fig. 5), which may have hindered the growth of Group A fish. Exposure to PFOS has been found to impact carbohydrate and lipid metabolism, processes that are essential for growth and development of organisms (Nayak et al., 2023). Embryonic growth and subsequent offspring development in zebrafish can be negatively impacted by PFOS exposure (M. Wang et al., 2011); however, levels of exposure were up 250 ng/g PFOS over five months in the study, a much higher

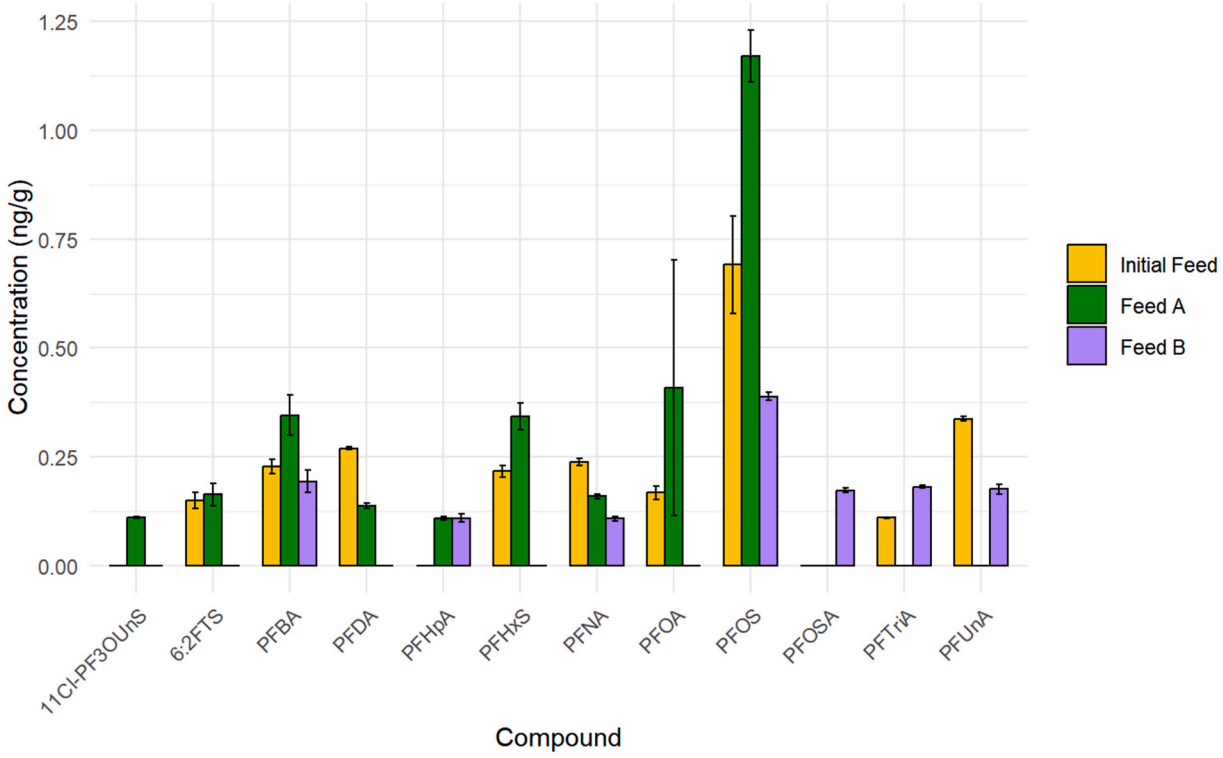

Fig. 5. PFAS concentrations (ng/g) observed in the initial feed, Feed A, and Feed B. Missing bars indicate that the PFAS was below the limit of detection in the corresponding feed. Error bars represent the standard error of the mean.

concentration and longer duration than this study. To our knowledge, no studies have been conducted to evaluate the effects of PFAS exposure on the growth of juvenile fish at concentrations comparable to those found in the feeds of this study.

Due to the short duration of the trial and relatively low concentrations of PFAS, such a difference in growth instead could be caused by nutritional differences in the feeds rather than differences in PFOS concentrations. Ikeogu et al. (2022) found that even slight differences in the nutritional components of fish feeds, such as crude protein, lipids, ash content, calcium, and phosphorus, had significant effects on the growth of juvenile catfish. Additionally, because only whole mass and standard-length measurements were taken, the present study could not fully evaluate whether the difference of PFOS in the feeds contributed to the observed growth disparities. Future studies should investigate how PFAS concentrations in aquaculture feeds, both naïve and modified, affect fish growth and pathology by extending the study duration to cover larval through adult stages, as well as whether there exists a critical point during growth and maturation at which PFAS accumulation begins as well as the potential impact of PFOS on the growth of aquaculture fish to inform best practices for feed formulation and sustainable aquaculture operations.

No relationship was observed between ration and fillet or liver PFOS concentrations, likely as a result of a complex interplay of biological growth and maturation, metabolic dynamics, variations in feeding practices, and other factors. It is important to note that controlling the amount of feed consumed by the fish proved to be a challenge, as the amount of leftover feed in the tanks varied. Anecdotally, there were often instances of leftover feed with the medium and high rations during the first half of the trial, leading to the need to drop the rations from 2 %, 4 %, and 6 % BW/day to 1 %, 2 %, and 3 % BW/day, to ensure all feed was consumed. Therefore, the lack of complete control over the feeding efficiency of the fish can be a potential explanation as to why no clear relationship was observed. It should also be noted that the present study only utilized juvenile red drum, but other fish species may have different nutritional requirements for optimal feeding efficiency, or different metabolic accumulation and depuration rates of PFAS (Galatius et al., 2013).

4.3. Dietary exposure estimates

Potential dietary exposures for both adults and children were estimated by calculating the estimated weekly intake of PFAS per kg BW. The calculations were based on an assumed intake of eight ounces of seafood per week, with 80.0 kg BW used for adult exposure and 15.0 kg BW used for child exposure. PFOS values used in these calculations reflected the median, lowest, and highest concentrations measured across the entire trial, with the highest values observed in the smallest fish. Overall, the dietary exposure estimates of this study did not exceed the EFSA's maximum TWI of 4.40 ng/kg BW/week (Table 2). However, body weight strongly influenced margin of safety. Based on our study's maximum PFOS concentrations in fish fillets, adults weighing 80.0 kg would need to consume 16 servings per week, whereas children weighing 15.0 kg would require just three servings (12 oz) per week to exceed the EFSA threshold. This demonstrates that children approach the TWI at much lower consumption rates compared to adults. Additionally, seafood consumption often varies depending on geographical location, cultural traditions, dietary practices, and more, meaning high-consumption individuals may face elevated PFOS exposure. However, the growth dilution effect observed in the study suggests that market-sized fish may have lower PFOS concentrations than the maximum levels measured here, potentially reducing dietary exposure. Future studies should further evaluate dietary risk in high-consumption groups.

It should be emphasized that the fish utilized in this study were not grown out to market size, and that PFAS accumulation can vary based on size and life stage. For instance, the initial fish of our study exhibited higher concentrations of PFOS than those of Group A, despite being given a diet lower in PFAS. Nonetheless, our results align with studies that have evaluated PFAS in fish that were obtained from supermarkets. Bedi et al. (2023) found that the TWI of the sum of PFOS, PFOA, PFHxS, and PFNA in both farm-raised and wild-caught fish from retail stores did

not exceed the EFSA threshold. Furthermore, farm-raised salmon, catfish, tilapia, and trout purchased from U.S. markets were found to have PFAS concentrations of less than 0.045 ng/g or not detected (Ruffle et al., 2020; Young et al., 2022). Thus, although the fish of this study were not market-sized, the results support existing research indicating that market-sized aquaculture fish generally exhibit low levels of PFAS contamination in fillets.

5. Conclusions

This study quantified PFAS in commercial aquaculture feeds and assessed their accumulation in aquaculture fish and potential dietary exposure to humans. Although the presence of measurable levels of PFAS in commercially available aquaculture feeds was confirmed, PFOS was the most prevalent compound in feeds and the only PFAS detected in fish fillets and livers, highlighting its persistence and bioaccumulative potential. No significant differences in PFOS concentrations in the feeds were observed based on protein source, crude protein content, or manufacturer. Fillet PFOS concentrations decreased over time, potentially due to growth dilution and maturation of detoxification systems, suggesting that steady fish growth may reduce PFAS burdens in edible tissues. Dietary exposure estimates for humans remained below the EFSA TWI. While these results may not directly represent market-sized fish, they are consistent with findings from other studies conducted on market-sized fish (Bedi et al., 2023; Ruffle et al., 2020; Young et al., 2022), though high-frequency seafood consumers, particularly children, may face elevated risk. Overall, the results of this study provide insight into PFAS contamination in aquaculture feeds that can help the aquaculture industry develop feeding practices for healthy and safe aquaculture products.

CRediT authorship contribution statement

Kelsey Blevins: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jessica Reiner:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Data curation, Conceptualization. **Aaron M. Watson:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Michael Janech:** Writing – review & editing, Conceptualization. **Ashley S.P. Boggs:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Data statement

The data supporting the findings of this study are available from the corresponding author, K. Blevins, upon reasonable request.

Disclaimer

These opinions, recommendations, findings, and conclusions are those of the authors and should not be construed to reflect the views or policies of the United States Government or any federal agency. Identification of certain commercial equipment, instruments, software, or materials does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products identified are necessarily the best available for the purpose.

Permits

Project #MML-2022-0352 was approved by the NIST Research Protections Office and determined to use only excluded specimens and/or data that have been determined to meet the criteria for vertebrate animal research *not involving live vertebrate animals* as defined in the policies for the NIST Humane Care and Treatment of Vertebrate Animals Program.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2026.116058.

Data availability

Data will be made available on request.

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