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Wide scope screening and target quantification of per- and polyfluoroalkyl substances (PFAS) in feed and feed ingredients to farmed salmonids

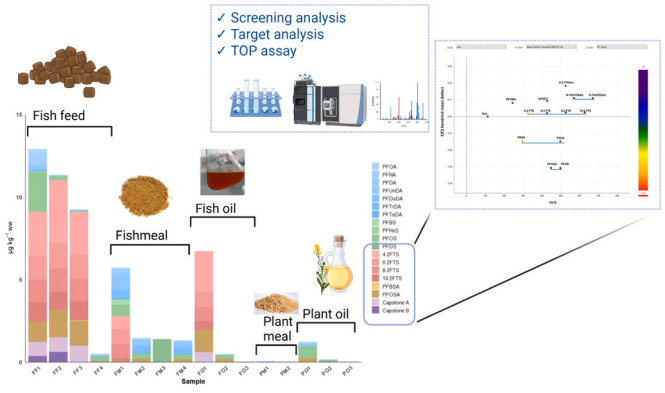
Aasim M. Ali, Marc HG Berntssen *, Veronika Sele, Stig Valdersnes

Institute of Marine Research (IMR), P.O. 1870 Nordnes, Bergen NO-5817, Norway

HIGHLIGHTS

- Other feed ingredients in addition to fish meal, such as fish and plant oil can be sources for PFAS in salmon feeds.
- PFAS precursors were present at higher concentrations than the legacy perfluoroalkyl acids in fish feeds.
- The "masked" precursors are relevant as potential indirect sources of persistent PFAS when fed to salmon.

GRAPHICAL ABSTRACT



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ABSTRACT

Fish meal (FM) is a recognized source of per- and polyfluoroalkyl substances (PFAS) in aquafeeds and following farmed seafood. Targeted determination and non-target wide scope screening was combined with total oxidizable precursor (TOP) assay in both marine- and plant-based feed ingredients used in farmed Atlantic salmon feed to Norwegian Atlantic salmon to assess pattern and source of PFAS contamination in the salmon seafood production chain. Of all main feed ingredients, FM had highest PFAS loads with 12 detected PFAS of which PFUnDA and PFOS as the dominant compounds (mean 0.34 and 0.32 μg kg^{-1}wet weight, respectively). Other feed ingredients, including fish oil (FO), plant oil (PO), and plant meal (PM), generally contain lower PFAS levels. However, PFOS was detected in all FO and PO samples, and as salmon feed contains mainly (>70 %) plant ingredients, they can significantly contribute to overall PFAS load in feed. More importantly, salmon feed contained high levels of PFAS precursors, at concentrations by far (up to two-fold) exceeding the legacy PFAS. As these precursors can be transferred into legacy PFAS, as confirmed by the TOP assay, the presence of these precursors can "mask" additional source and loads of PFAS in farmed salmon.

* Corresponding author.
E-mail address: marc.berntssen@hi.no (M.H. Berntssen).

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic organic chemicals widely used in many industrial applications such as surfactants, fluorinated polymers, and fire extinguishing foams [1]. Buck et al. (2011) [2] defined PFAS as substances containing a perfluoroalkyl moiety and hydrophilic head, encompassing thousands of known compounds. With the re-definition of PFAS by Organisation for Economic Co-operation and Development (OECD) in 2021, encompassing all molecules with one fully fluorinated methyl or methylene carbon (without H or other halogens attached), the number of known PFAS abruptly increased to more than 7 million compounds [3]. Since the 1990s, long-chain perfluoroalkyl acids (PFAAs), specifically perfluoroalkyl carboxylic acids (PFCAs, ≥7 perfluorinated carbons), perfluoroalkanesulfonic acids (PFSAs, ≥6 perfluorinated carbons), and their precursors, particularly perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) have gained global attention from scientists, regulators, and the public[4]. Importantly, after over 50 years of production, PFOS, as well as PFOA, and perfluorohexane sulfonate (PFHxS), their salts and related compounds are listed under the Stockholm Convention on Persistent Organic Pollutants (POPs). Also, the European Union (EU) has made legislative efforts to restrict their use as they can be harmful to human health and the environment [5,6]. Despite the restricted use of PFAS nowadays, the decades long use of these chemicals have made them ubiquitous in the environment, and particularly in the aquatic environment [7]. The long-chain perfluoroalkyl acids, have been found to bioaccumulate in the protein-rich tissues of marine organisms, including fish [8,9]. There is a growing concern about the health risks posed by perfluoroalkyl substances, as parts of the European population are surpassing the established tolerable weekly (TWI) intake for certain PFAS [10]. Food of animal origin, and especially seafood, is an important contributor to the human dietary exposure to PFASs [11–13]. Adverse health end-points for PFAS exposure for humans includes cholesterol disturbance [12,14], liver damage [12,15], development disorder and impaired immunology, as the most critical end-point [12,16]. In 2020, the European Food Safety Authorities (EFSA) performed a risk assessment for human exposure of four PFAS (PFOS, PFOA, perfluorononanoic acid [PFNA], PFHxS) [12], and consequently maximum limits (ML) were set for these four PFAS (4-EFSA PFAS) in food products, including seafood [17].

The global production of cultured seafood is exceeding the global wild capture of fisheries as the main food source from the sea[18]. Formulated aquafeeds are considered as the main source for POPs in farmed Atlantic salmon (*Salmo salar*) [19]. Global pollution and bioaccumulation of POPs in the marine food-webs have caused elevated levels in feed ingredients obtained from marine fish stocks [7]. For the protein-philic PFAS, fishmeal produced from wild marine fish stocks is one of the main sources for PFAS in farmed animals [20–22]. Farmed carnivorous fish species such as Atlantic salmon (*Salmo salar*) have a relatively high inclusion of fish meal in their formulated feed, although fish meal to a large degree has been replaced with plant ingredients the last decades in developing more sustainable salmon feeds (from 33.5 % use of fish meal in 2000–12.1 % in 2020) [23]. As feed ingredients can be a main source for PFAS in food products of animal origin, the European Commission (EC) requested member states to provide more occurrence data on the levels of PFAS in feed and feed ingredients in order to set appropriate MLs for PFAS at the start of the food production chain [10]. Several earlier studies have surveyed the level of selected target PFAS in fishmeal, reporting on the pattern of PFAS in fish meal that have bioaccumulated in the marine food webs [21,22]. Few studies have reported on the PFAS composition in additional aquafeed ingredients than fishmeal. Although PFAS are generally considered protein-philic, PFAS has also been reported in fish oils [24], which consist of 10 % of the high energy salmon feeds [23]. In animal feeds that are used in laboratory toxicity testing (e.g. for zebra fish), plant ingredients (e.g. soy products or wheat) were found to be an important source for short-chained PFAS, whereas marine feed ingredients (e.g. fish meal or shrimp) contained more long-chain PFAS [25]. Plant-based feed ingredient consist of up to 73 % of currently produced salmon feeds [23], and little is known on the contribution of commercial plant-ingredients to the PFAS pattern and levels to commercial salmon feeds and hence farmed seafood[26].

In addition to the known PFAAs, there is growing attention on polyfluoroalkyl substances known as ‘‘PFAS precursors’’ in fish meal or marine ingredients such as perfluorooctane sulfonamide (FOSA) [27] or fluorotelomer sulfonates (6:2 FTS) [26]. Plant feed ingredient such as hay and barley grown on PFAS contaminated soil also contained several PFAS precursors [28]. These precursors can transform through various biological pathways into well-known PFAAs, thereby increasing their load in farmed animals [29,30]. Many of the PFAS precursors are usually not included in routine PFAS analyses, however, knowledge about their presence in feed ingredients is of importance when assessing PFAAs feed-to-food transfer since the precursors are an extra “masked” source, contributing to the final PFAS level in the farmed animal [29]. To our knowledge, there is limited knowledge about the presence of PFAS precursors in both processed marine and plant-based feed ingredients, and salmon feed. Most PFAS analyses target a specific set of PFAAs and their known precursors. These analyses typically involve the use of high-performance liquid chromatography (HPLC) coupled with either a low-resolution or a high-resolution mass spectrometer (LR/HRMS) [22, 27,31–33]. However, accurate identification and quantification of PFAS with these methods require analytical standards, which currently exist for fewer than 100 of the many PFAS. Therefore, there is an increased interest in using non-targeted analytical approach for assessing PFAS in biota to identify additional suspected and unknown PFAS [34,35]. To quantify the unidentified fraction of PFAS in, several techniques for measuring bulk organofluorine have been developed, though their selectivity and inclusivity differ, with particular emphasis on [36] total oxidizable precursor (TOP). TOP assay is the most selective among bulk organofluorine methods, as it specifically targets unidentified oxidizable PFAS precursors that are not covered by targeted analyses, by converting them into analyzable PFCAs [37]. Further, with the development of HRMS and the capability for "suspect screening" using non-target analytical methods, more extensive lists containing thousands of PFAS have become accessible [38,39]. In addition to precursors, current PFAS are being replaced with new PFAS-like substances that will contribute to the PFAS load in animal feed. However, beyond the 4–17 PFAS emphasized by EFSA, and previously studied by a target approaches, little information exists on the broader presence of suspected and non-target PFAS that could potentially be present in fish meal or other feed samples.

The present paper extends on earlier studies reporting on the presence of target PFAS in fish meal, which is considered the main source for PFAS in animal feeds [21,22,27], by employing a comprehensive strategy to identify and quantify PFAS in Norwegian salmon feeds, plant- and fish-based ingredients, a list of 28 PFAS, including novel precursors PFAS, are determined using ultra-HPLC coupled with triple quadrupole mass spectrometry (QqQ/MS). Additionally, suspect and non-target wide-scope screening is performed utilizing a suspect list of approximately 49,000 PFAS with UHPLC-HRMS. The study also examines the role of unidentified precursors in the formation of PFAA, utilizing an optimized TOP assay method.

2. Materials and methods

2.1. Chemicals and reagents

Fourteen perfluoroalkyl carboxylic acids (PFCAs), specifically C2 to C14, C16, and C18 chain lengths. Additionally, ten perfluoroalkyl sulfonates (PFSAs) analyzed included C1-C4, C6-C10 and C12. The analysis also included important five PFAS precursors, namely perfluorooctane sulfonamide (FOSA) and fluorotelomer sulfonates (i.e., 4:2FTS, 6:2FTS,

8:2FTS, 10:2FTS), as well as four emerging PFAS replacements, i.e, 3H-Perfluoro-3-[(3-methoxy-propoxy)propanoic acid] (ADONA), hexafluoropropylene oxide dimer acid (HFPO-DA), 9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS), 11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUnDS). All PFAS with their isotopically labelled internal standards with high purity (>98 %), were purchased from Wellington Laboratories Inc. (Ontario, Canada). Detailed structural information and supplier details for all targeted compounds are provided in Table S1.

2.2. Samples

The present study aims to implement a matrix specific targeted and wide scope screening of PFAS and precursors for the main ingredients used in commercial salmon feeds including both plant and fish-based protein and fatty-acid feed sources. The total yearly volume of Norwegian commercial salmon production is approximately 1.5 million tonnes, using approximately 2 million tonnes of feed ingredients with 0.5 million tonnes of marine feed ingredients and 1.4 million tonnes of plant origin [23]. The sample size in the present study does not reflect the overall yearly production volume of commercial produced salmon feed in Norway, however the feed ingredients selected and hence matrices for analytical method development are representative for the type of feed ingredients commercially used. For plant ingredients, soy and wheat are the main protein sources and rapeseed/camelina are the main oil sources [23]. Fish meal is considered as the main source for PFAS in aquafeeds [21,22], and most of the fish meal is of North Atlantic origin [23]. The catch or harvest location and crop-based selected are given in Table 1.The sample types selected are considered the main likely dietary sources of PFAS in Salmon aquaculture production, and include commercially produced Norwegian salmon feeds and representative commercially feed ingredients that comprise the main part of commercial salmon feeds, including plant meal, plant oil, fish meal and fish oil [23]. A total of fifteen randomly collected samples, including commercial feed samples and feed ingredients, from the Norwegian monitoring program for fish feed, were selected for analysis. The feeds and feed materials have been sampled by the Norwegian Food Safety Authorities (NFSA) at randomly selected feed production facilities in Norway [24]. The samples include four salmonid feed samples, four fish meal samples, three fish oils, three plant oils, and two plant meals. As the samples are randomly selected, the feed ingredients are not directly related to the selected feed samples.

Table 1
Overview of commercial feed ingredients assessed in the present study that are representative for the type of feed ingredients currently used in Norwegian commercial salmon feeds including samples with from different geographic locations and different crops.

marine	catch location			catch location	species included
fish meal 1	Chile/ South Pacific		fish oil 1	Mexico	Sprat, anchovy, herring
fish meal 2	Faroe Islands/ North Atlantic Ocean		fish oil 2	Oman	Sardines
fish meal 3	Iceland/North Atlantic Ocean		fish oil 2	Peru, India, Mauritania	Anchovies
fish meal 4	Norwegian sea North Atlantic Ocean				
Plant	harvest location	crop included		harvest location	crop included
plant meal 1	Brazil	Soya protein (SPC)	plant oil 1	Belarus	Rapeseed oil
plant meal 2	Italy	Wheat gluten	plant oil 2	Belarus	Camelina oil
			plant oil 3	Germany, Ukraine, Belgium	Mix of plant oil oils

2.3. Sample preparation

Samples were extracted using the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method. This method was adapted with modifications from a previously established procedure [37,40,41]. The salt amount used is consistent with these methods and was appropriate for extracting PFAS from the samples investigated. Two grams of each sample material were weighed in duplicates into 50 mL polypropylene tubes. Fifty microliters of internal standard (ISTD) of 100 ng/mL concentration solution were spiked. Following the spiking, 5 mL of Milli-Q water and 10 mL of acetonitrile (MeCN) were added to each tube. The samples were vortexed for about 20 s and subjected to ultrasonic extraction for 30 min at room temperature. After sonication, 6 g magnesium sulfate ($MgSO_4$) and 1.5 g sodium chloride (NaCl) (Supel™ QuE, Non-Buffered, Tube 2. Merck, Darmstadt, Germany) were added to each tube to facilitate phase separation, followed by vortexing for an additional 20 s. The samples were then centrifuged at 4000 rcf for 5 min. A total of 6 mL of the supernatant was carefully transferred to new 15 tubes containing 300 mg Supelclean™ PSA, 150 mg Supelclean™, ENVI-Carb™, 900 mg $MgSO_4$ (Supel™ QuE PSA/ENVI-Carb™ Tube 3; Merck, Darmstadt, Germany) and vortexed again before a second centrifugation step. Finally, 1.5 mL of the clear supernatant was transferred into 2 mL vials for subsequent instrumental analysis.

2.4. Target analysis of feed and feed ingredients

Quantification of targeted PFAS was performed using LC-MS/MS (triple quadrupole). Conventional PFAS were separated using a ZORBAX Extend Plus C18 column (RRHD 2.1 × 100 mm, 1.8 µm, Agilent Technologies, USA), while ultrashort-chain trifluoroacetic acid (TFA) was analyzed using a hybrid HILIC/ion-exchange column (Raptor Polar X; 50 × 2.1 mm, 2.7 µm, Restek Corp., Bellefonte, PA). Both chromatographic columns were coupled to an Agilent 1290 HPLC system (Agilent Technologies, Waldbronn, Germany) and compounds were detected using an Agilent 6495B triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source and iFunnel technology. PFAS were monitored using dynamic multiple reaction monitoring (dMRM) transitions (Table S2 and S3). For conventional PFAS, the mobile phase consisted of 5 mM ammonium acetate in water as the aqueous phase and 5 mM ammonium acetate in methanol as the organic phase, with a flow rate of 0.2 mL/min. The column temperature was set to 40°C, and the gradient program is detailed in Table S4. For ultrashort-chain PFAS, the mobile phase was a mixture of 10 mM ammonium formate and 0.1 % formic acid in water (A) and 0.1 % formic acid in 95:5 acetonitrile: isopropanol (B) (15:85, A:B), see Table S5. The flow rate was 0.3 mL/min for 4 min, and the column temperature was maintained at 40°C. MassHunter Quantitative Software (v8.0) was used for data processing and quantification.

2.5. High-resolution mass UHPLC-Q-Orbitrap HRMS screening work flow

Suspect and non-target screening, as well as confirmation of compounds detected with only one single MRM transitions, was performed using ultra-high-performance liquid chromatography (UHPLC) (Vanquish Flex UHPLC system, Thermo Fisher Scientific, USA) coupled with a Thermo Scientific™ Orbitrap Exploris™ 120 high-resolution mass spectrometer equipped with a heated electrospray ionization (H-ESI) source (UHPLC-Q-Orbitrap HRMS), operated in negative ionization mode. Chromatographic separation was achieved using a 150 × 2.1 mm, 2.7 µm AQ-C18 Poroshell column (Agilent Technologies, USA). Instrumental and chromatographic parameters for the UHPLC-Q-Orbitrap

HRMS analysis are summarized in Tables S7 and S8. Chromatographic separation was performed using a water/methanol gradient with 2 mM ammonium acetate at a constant flow rate of 0.25 mL/min. The system operated in negative ionization mode with data-dependent acquisition (DDA), and MS^1 and MS^2 scans were acquired at resolutions of 60,000 and 30,000 (FWHM at *m/z* 200), respectively. A stepped higher-energy collisional dissociation of 20, 60, and 100 V was used for fragmentation. HRMS raw data were processed using Thermo Scientific™ TraceFinder™ 5.1 software, which contained a curated library of target analytes, including molecular formulas, adducts, retention times, and characteristic fragment ions. Mass error tolerance was set to 5 ppm for both MS1 and MS2, and isotopic profile matching was set to 70 %. Calibration curves and the integration of native compounds with their respective internal standards were manually verified to ensure accuracy.

Suspect and non-targeted screening of PFAS was performed using Compound Discoverer 3.3.SP3 (Thermo Fisher Scientific) with a modified version of the PFAS-optimized "Untargeted PFAS Workflow." The workflow included retention time alignment, detection of unknown compounds based on selected adduct ions ($[M+Cl]^-$, $[M+FA-H]^-$, $[M-H]^-$, $[M-H+HAc]^-$, $[M-H-H_2O]^-$) with a minimum intensity of 1000, and compound grouping prioritizing $[M-H]^-$. Elemental compositions were predicted, followed by gap filling and background subtraction using procedural blanks. Compound annotation was conducted using multiple sources, including the Thermo Scientific™ mzCloud™ spectral library, and a computational mass spectrometry library [39]. In addition, a mass list 'PFAS-suspect-DB-Dunke' which contains 48896 PFAS was utilized [39]. Additionally, the PFAS workflow included three extra data processing steps for further confirmation: (a) 'Compound Class Scoring', which identifies PFAS using 805 diagnostic fragments from FluoroMatch Suite [42] and 16 exclusive PFAS fragments from the Fine Signature Fragment database; (b) 'Molecular Network Visualization', which groups related PFAS based on fragmentation similarity; and (c) 'PFAS Scripting Node', which estimate the number of carbon atoms estimation from the predicted elemental composition, utilizing the height of the first isotopic peak (M+1) and its corresponding monoisotopic peak(M) [43]. In turn, two additional ratios are calculated: molecular mass divided by number of carbon atoms and mass defect divided by number of carbon atoms (m/C and md/C, respectively). C was calculated based on the ration of abundance of the first isotopic peak to the abundance ratio of the corresponding monoisotopic peak (M + 1)/M; $^{13}C/^{12}C$.

For feature prioritization, in addition to the common filters used for data reduction in HRMS (i.e. background subtraction and peak shape quality), PFAS-specific filters were used, such as CF_2 Kendrick mass defect [43] chemical transformations, as well as fragmentation and formulae that contain more than two fluorine atoms were used. Further nontargeted strategy was adapted from Kaufmann et al. (2022) [43] which can find any PFAS of F/C ratio > 1. In this strategy, the two ratios m/C and md/C calculated are plotted via PFAS cluster on the bottom right of the plot as shown in Figure S1. Finally, confidence level in the identification of PFAS is clarified following the PFAS specific confidence scale [44]. This scale ranges from the highest confidence level 1a "Confirmed by Reference Standard," to least confidence level 5 "Exact Masses of Interest," which are prioritized by suspect list or the PFAS filtering tools. However, in the current study only PFAS identified at level 1 and 2 are reported. Further methodological details are available in previously published studies [31,41].

2.6. Total oxidizable precursor (TOP) assay

A modified version of the TOP assay, originally described by Houtz and Sedlak [37], was applied in this study. For TOP analyses, two new replicates of 2 g of each sample material were placed into 50 mL polypropylene tubes. No ISTDs spiking was performed before extraction. To each tube, 5 mL of Milli-Q water and 10 mL of MeCN were added, followed by vortexing and ultrasonic extraction for 30 min. The same amount of NaCl and MgSO4 were then added, and the tubes were vortexed. Samples were centrifuged at 10,000 rcf for 5 min, and 8 mL of the supernatant was transferred to new 15 mL tubes for clean-up as discussed above. From the supernatant 3 mL was transferred into two separate 15 mL polypropylene tubes. The supernatants in both tubes were evaporated to dryness. To this tube, 4 mL of potassium persulfate ($K_2S_2O_8$, 4.7 g/100 mL) and 2 mL of sodium hydroxide (NaOH, 20 g/100 mL) were added. In the other tube, 6 mL of Milli-Q water was added. Both tubes were sonicated for 15 min and then incubated in a water bath at 85°C overnight (from 11:00–06:30). After incubation, the samples were neutralized with 800 µL of concentrated hydrochloric acid (HCl) and 800 µL of Milli-Q water was added to the tube without the TOP assay treatment. Both tubes were then spiked with 50 µL of PFAA internal standard (IS) of 50 ng/mL concentration. Finally, the samples were vortexed, and 1.5 mL was transferred to LC-MS vials for analysis.

2.7. Quality control and quality assurance targeted analysis and TOP assay

The apparent recoveries of PFAS in the targeted matrices were calculated by representative matrix from the real sample batches with a native PFAS mixture (1 ng). These spiked samples were then extracted using the same QuEChERS method applied for the actual sample analysis, as PFAS-free samples were not available. Empty tubes (n = 6) were used as procedure blanks and were treated in the same manner as real samples. To control the oxidation process in TOPA method, an empty tube was spiked with isotopically labeled perfluorooctanesulfonamide ($^{13}C_8$-FOSA), then the methanol from the spiking solution was evaporated, and the residue was oxidized using the same method. An Agilent perfluorinated compound (PFC)-free conversion kit was installed on Agilent 1290 with PEEK and stainless steels components including a PFAS trapping column, which reduces PFAS contamination from the HPLC system [45]. To control the carryover effect, methanol was injected with every 10 samples. PFAS concentrations that were above the limit of detection (LOD) and below the limit of quantitation (LOQ) were unchanged. The LOQ for each PFAS was determined based on apparent recoveries at spiked concentrations of 0.03, 0.05, 0.25, 0.5, and 5 ng/g using cod fish liver samples. For some PFAS, the LOD and LOQ were calculated using the standard deviation of the response and the slope as recommended by the international conference on harmonization (ICH) and the values are given in Table S9. Using the EURL guidance, recoveries between 65–135 % were considered acceptable, and the lowest concentration meeting this criterion was assigned as the LOQ.

The apparent recoveries of PFAS in the targeted matrices were calculated by spiking two replicates of representative matrix from the real sample batches with a native PFAS mixture at a concentration of 1 ng. These spiked samples were then extracted using the same QuEChERS method applied for the actual sample analysis, as PFAS-free samples were not available. This approach reflects the recovery performance under real sample conditions and ensures that the spiking procedure mirrors the matrix complexity encountered during real sample analysis. Most PFAS demonstrated acceptable recoveries, defined as 65–135 % based on guidance from the EURL for halogenated POPs in feed and food (2024) for monitoring purpose. However, certain compounds exhibited recovery values outside the acceptable range. For instance, PFBA showed an elevated recovery in fish meal, fish oil, and plant meal, and similar over-recoveries were observed for PFTrDA (186.6 ± 8.9 %) in fish meal and L-PFDoDS (140.2 ± 2.4 %) in fish oil. These deviations from the acceptable range suggest potential matrix effects or issues with extraction efficiency in these matrices. Similarly, PFPeA (in fish feed) and PFHxA (in fish feed and plant meal) in fish feed displayed unacceptable recovery from some matrices, indicating a potential loss or poor extraction from this matrix. Capstone A, Capstone B, and HFPO-DA were not detected at the spiked level of 1 ng, indicating incomplete recovery. Consequently, their reported concentrations should be regarded as semiquantitative. Despite these outliers, the

majority of PFAS recoveries were within the acceptable range across matrices, underscoring the reliability of the QuEChERS method for most compounds tested. To evaluate the efficiency of the applied TOP assay, duplicate samples from each matrix were spiked with 10 ng of isotopically labeled perfluorooctane sulfonamide ($^{13}C_8$-PFOSA) and processed using the same protocol. Complete conversion of $^{1\ 3}C_8$-PFOSA was observed in fish meal, fish feed, plant meal, and plant oil matrices. However, the fish oil matrix exhibited incomplete oxidation of $^{1\ 3}C_8$-PFOSA at the same concentration.

3. Results and discussion

3.1. Targeted analysis

3.1.1. Fish meal

The long-chain perfluoroalkyl acids, have are known to bioaccumulate in the protein-rich tissues of marine organisms, including fish [8,9]. Consequently, fish meal from wild marine fish is one of the main sources for PFAS in formulated feeds for farmed animals [20–22]. While fish meal use in feeds for carnivorous fish species like Atlantic salmon (*Salmo salar*) has decreased from 33.5 % in 2000 to 12.1 % in 2020 due to the introduction of more sustainable plant ingredients [23], it remains a likely source for significant PFAS in salmon feeds. Randomly selected commercial fish meal (FM) used in Norwegian salmon feed production, were subjected to targeted analysis. A quality control validated method was used to quantify 28 PFAS compounds, including 13 PFCAs with chain lengths ranging from C4 to C14, C16, and C18, along with seven PFSAs (C4, C6 to C10, and C12). The analysis also incorporated six precursors including PFOSA and four fluorotelomer sulfonates (4:2 FTS, 6:2 FTS, 8:2 FTS, 10:2 FTS), and six emerging PFAS replacements: ADONA, HFPO-DA, 9Cl-PF3ONS, and 11Cl-PF3OUdS. Table 2 shows the concentration of 12 PFAS (μg kg $^{-1}$ wet weight (ww)) that were detected in the present study and compared to concentrations reported in earlier surveillance studies on fish meal [21,22]. Of the 28 targeted compounds, ten PFCA/PFSAs were detected or quantified > LOQ in fish meal, these included starting with highest concentration, perfluoroundecanoic acid (PFUnDA) (0.34 μg kg^{-1}), PFDS (0.34 μg kg^{-1}), PFOS (0.32 μg kg^{-1}), PFTrDA (0.28 μg kg^{-1}), PFNA (0.10 μg kg^{-1}), PFBS (0.08 μg kg^{-1}), PFDA (0.07 μg kg^{-1}), PFDoDA (0.07 μg kg^{-1}) PFTeDA (0.05 μg kg^{-1}), PFOA (0.02 μg kg^{-1}) (Table 2). [21,22,27]. The pattern of PFAS was in general similar to those earlier reported with the long chain PFOS and PFUnDA in highest concentration, followed by PFTrDA, PFNA, PFDoDA, PFDA (Table 2). The current study analyzed four fish meals, including three sourced from the Northern Hemisphere (Faroe Islands, Iceland, and Norwegian Sea) and one from the Southern Hemisphere (Chile) (Table 2). The levels of the dominant PFOS and PFUnDA in European fish meals in the present study were considerably lower than those previously reported for European fish meals. In this study, the PFOS+PFUnDA concentration was 0.66 μg kg^{-1}, compared to 3.95 μg kg^{-1} in earlier reports of European

Table 2
Concentrations (μg kg^{-1}) and limit of quantifications (LOQ) of PFAS in four randomly selected fish meals used in Norwegian Atlantic salmon feed production. For each commercial fish meal catch location is given. The results from this study are compared with earlier global studies on fish meal (Li et al. 2019)[21] and Danish used fish meals (Suominen et al. 2011)[22]. Concentrations for this study are given as "lower bound" (LB) with values <LOQ are set equal to zero and "upper bound" (UB) with values <LOQ set equal to LOQ. ND= not detected. Abbreviations for PFAS are given in the main text.

			PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
This study	*catch location*								
fish meal 1	Chile/ South Pacific		0.09	0.23	0.18	0.75	0.16	0.47	0.07
fish meal 2	Faroe Islands/ North Atlantic Ocean		ND	0.10	0.04	0.34	0.07	0.34	0.05
fish meal 3	Iceland /North Atlantic Ocean		ND	0.08	0.07	0.28	0.04	0.30	0.07
fish meal 4	Norwegian Sea North Atlantic Ocean		ND	ND	ND	ND	0.01	ND	ND
		method LOQ	0.05	0.03	0.25	0.03	0.05	0.25	0.05
Li et al. 2019	*								
fish meal (n = 45)	South America	min	0.04	0.04	0.05	0.05	0.06	0.07	
		max	3.25	4.80	3.25	10.10	2.29	7.55	
		median	0.15	0.22	0.05	0.35	0.06	0.07	
fish meals (n = 5)	European	min	0.04	0.04	0.05	0.05	0.06	0.07	
		max	2.29	3.01	1.84	9.7	1.99	8.1	
		median	0.15	1.55	0.73	2.39	0.34	0.65	
		method LOD	0.02	0.02	0.03	0.03	0.03	0.04	
Suominen et al. 2011	**								
fish meal (n = 10)	production Northern Europe	min		< 0.5	< 0.5	~ 0.6		~ 0.2	< 0.5
	Denmark, Poland, Norway, Island	max	~ 0.5	< 0.5	< 0.5	~ 2	< 0.5	~0. 2	< 0.5
		metod LOQ 0.04–0.09							

			PFBS	PFHxS	PFHpS	PFOS	PFDS	total LB	total UB
This study	*catch location*								
fish meal 1	Chile/ South Pacific		0.31	ND	ND	0.67	ND	2.91	2.94
fish meal 2	Faroe Islands/ North Atlantic Ocean		ND	ND	ND	0.33	ND	1.26	2.68
fish meal 3	Iceland/North Atlantic Ocean		ND	ND	ND	0.30	ND	1.13	1.30
fish meal 4	Norwegian sea North Atlantic Ocean		ND	ND	ND	ND	1.38	1.38	2.38
		method LOQ	0.03	0.03	0.03	0.25	0.03		
Li et al. 2019	*								
fish meal (n = 45)	South America	min	0.04	0.04		0.22		1.06	
		max	1.48	14.6		27.8		42.1	
		median	0.04	0.55		1.67		4.78	
fish meals (n = 5)	European	min	0.04	0.27		0.02		2.47	
		max	1.36	14.7		27.1		45.3	
		median	0.14	1.94		1.11		16.7	
		method LOD	0.02	0.03		0.02			
Suominen et al. 2011	**								
fish meal (n = 10)	Production Northern Europe	min		< 0.5				~0.9	
	Denmark, Poland, Norway, Island	max		< 0.5		~ 15		~20	
		method LOQ 0.04–0.09							

*only PFAs which are detected at a frequency of 50 % are included in the study (Li et al., 2019) [21]
**Values estimated from a figure, values with less than 0.5 ng/g are defined as < 0.5 μg kg^{-1} (Suominen et al., 2011) [22]

produced fish meals that were used in Chinese feed factories from 2012 to 2014 [21]. In a 2019 global assessment of 17 selected PFAS in fish meal, fishmeal that originated from the Northern Hemisphere were significantly higher in PFAS levels than those from the Southern Hemisphere [21]. In contrast, one of the fish meals in the present study was from the Southern hemisphere (South America, Pacific Ocean) and contained higher indicator PFOS+PFUnDa levels (1.4 μg kg^{-1}) than the three fish meals from the Northern hemisphere (Northern Europe, North Atlantic Ocean). In an earlier study, ten commercial European fish meals showed only PFOS, PFUnDA and PFTrDA levels above the detection limit with a large variation in levels among these fish meals with total PFAS levels ranging from 0.9 to 20 μg kg^{-1} [22]. The present study included a limited number of commercial fish meals currently included in Norwegian produced salmon feeds, showing a large variation in PFAS concentrations among the fish meals. For example, one fish meal had no quantifiable levels of the dominant "indicator" PFOS and PFUnDA, but had relatively high levels of PFBS, normally not detected or at very low levels in other fish meals (Table 2). For another fish meal, PFOA was quantified but not in the other fish meals, while earlier surveillance studies reported PFOA in most fish meals [21,22] (Table 2). This warrants the need for more detailed studies on the geographic origin and fish species used for the production of fish meals to assure potential sourcing of fish meals with low PFAS levels. Fish meal used in salmon feeds are world-wide procured and knowledge on PFAS distribution in fish meal after geographic origin, seasonal variation, and species of fish stocks is important in sourcing fish meal with low environmental PFAS levels, thus mitigating the introduction of PFAS in the salmon food production chain. The differences observed between PFAS levels in earlier samples (2012–2014) [21] and the current study may be influenced by temporal changes, including reductions due to phase-outs and increased regulations. This highlights the importance of updating regulations to capture changes of PFAS levels in fish meals over time.

3.1.2. Other feed ingredients

Most studies on aquafeeds have focused on fish meal as the dominant source of PFAS, however few studies have reported on PFAS in the other feed ingredients to aquafeeds. Fish oil contributes typically for 10 % in high energy salmon feeds [23], and salmon feeds generally comprise up to 73 % plant-based feed ingredients, i.e. soy protein concentrate, wheat gluten, wheat and rapeseed oil [23]. In the present study, the targetd analysis used for fish meal was also applied to randomly selected commercial feed ingredients that comprise 83 % of todays salmon feed, including both fish oil (FO) and plant meal (PM) and plant oil (PO). In general, the levels of PFAS were lower in these feed ingredients than when compared to fish meal, however, not devoid of PFAS. For example, both fish oil and plant oil contained PFOS with a large variation in concentrations among oil sources, with one plant oil having particularly high concentration of PFOS (0.71 μg kg^{-1}), higher than those registered for fish meal (Table 3). Surprisingly, both fish oil and plant oil contained low levels of long-chained PFOS which is a environmental PFAS mostly associated with bioaccumulate in the protein-rich tissues of marine fish with elevated levels in fish meal [8,9]. Both fish and plant oils used in animal feeds, are pressure extracted and contain minor protein fraction that might contribute to the oil PFAS contamination. For plant oils, PFAS contaminated soil is a well-known source for PFAS, including long-chain PFOS [28], and several techniques have been developed to remove PFAS from contaminated soils [46] which can mitigate PFAS loads in crop. As commercial salmon feed consist 20 % of PO, this feed ingredient could be a considerable source for PFOS in salmon feeds. In PM samples, PFAS levels were predominantly below detection limits, except for minor levels of PFDoDA and PFTrDA, which were detected at the LOQ. In a survey on PFAS in animal feeds used in laboratory toxicity testing (e.g. for zebra fish), a clear distinction between feeds based on plant ingredients (e.g. soy products or wheat) or aquatic animals (e.g. fish meal or shrimp) ingredients was seen [25]. The plant-based experimental diets contained mostly short-chain PFAS while feed containing animal-based ingredients contained longer chain PFAS [25]. This was thought to be consistent with a higher bioaccumulation of longer chain PFAS in the marine food web [9] compared to the preferential bioaccumulation of short-chain PFAS in plants [25,47]. In plant-based feed (hay and barley), grown on land polluted with industrial waste with high concentrations of PFAS, short-chain PFAAs and long-chain PFOS were the most abundant PFAS [28]. In the present study, short-chain PFAS such as PFBA, PFBS and PFHxS were not detected in either plant meal or plant oil at LOQ < 0.03 μg kg^{-1}. In addition to the quantified PFAS, various ultra-short chain PFAS were also screened in the feed samples. Among them, only trifluoracetic acid (TFA) was detected and semi-quantified. The semi-quantification data of TFA showed high variability across sample types, likely due to differences in processing and material composition. Fishmeal exhibited TFA concentrations between 2.3–12.7 μg kg^{-1} ww, while most FO and PO samples had non-detectable levels corroborating the water-soluble properties of this ultra short chain PFAS. Plant meal samples contained 1.0–1.4 μg kg^{-1} ww, thus indicating no specific differentiation in more ultra short chain PFAS between marine or plant derived feed ingredients. The findings in the present study indicate that PFAS contamination in fish feed is primarily associated with marine-derived ingredients (FM) rather than plant-based sources. However, of the few

Table 3
Concentrations μg kg^{-1} and limit of quantifications (LOQ) of PFAS in four randomly selected fish oil, plant meals and plant oils used in Norwegian Atlantic salmon feed production. Concentrations for this study are given as "lower bound" (LB) with values <LOQ set equal to zero. ND= not detected. Abbreviations for PFAS are given in the main text.

	catch/harvest location	*species included*	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
fish oil 1	North Atlantic	Sprat, herring and mackerel	ND	ND	ND	ND	0.005	ND	ND
fish oil 2	Oman	Sardines	ND	ND	ND	ND	0.01	ND	0.005
fish oil 2	Peru, India, Mauritania	Mix of oils from Engraulis	ND	ND	ND	ND	0.01	ND	ND
plant meal 1	Brazil	Soya protein concentrate (SPC), > 60 %	ND	ND	ND	ND	0.005	ND	0.035
plant meal 2	Italy	Wheat gluten	ND	ND	ND	ND	0.015	ND	ND
plant oil 1	Belarus	Rapeseed oil	ND	ND	ND	ND	0.005	ND	ND
plant oil 2	Belarus	Camelina oil	ND	ND	ND	ND	ND	ND	0.035
plant oil 3	Germany, Ukraine Belgium	Mix of oils	ND	0.13	0.04	0.08	0.015	ND	ND

	catch/harvest location	*species included*	PFBS	PFHxS	PFHpS	PFOS	PFDS	total LB
fish oil 1	Mexico	Sprat, anchovy, herring and mackerel	ND	ND	ND	ND	ND	0.005
fish oil 2	Oman	Sardines	ND	ND	ND	ND	ND	0.015
fish oil 2	Peru, India, Mauritania	Mix of oils from Engraulis,	ND	ND	ND	0.26	ND	0.27
plant meal 1	Brazil	Soya protein concentrate (SPC), > 60 %	ND	ND	ND	ND	ND	0.04
plant meal 2	Italy	Wheat gluten	ND	ND	ND	ND	ND	0.015
plant oil 1	Belarus	Rapeseed oil	ND	ND	ND	ND	ND	0.005
plant oil 2	Belarus	Camelina oil	ND	ND	ND	0.06	ND	0.095
plant oil 3	Germany, Ukraine Belgium	Mix of oils	ND	ND	ND	0.715	ND	0.98

plant ingredients included in the present study a large variation exists in PFAS cocentrations, and the relative high inclusion level of plant material in salmon feeds, warrents the need for more extensive surveillance of plant-based feed ingredients, in particular PO to identify additional sources of one of the domiant PFOS as one of the dominant PFAs in aquafeeds.

3.1.3. Feed

Targeted analyses of PFAS in fish feed showed PFOS as the most dominant PFAS (0.73 μg kg^{-1}), followed by PFNA (0.20 kg), and to a lesser extent PFOA and PFUnDA (0.07 μg kg^{-1}), and PFDA, PFTrDA, PFDoDA, PFTeDA and PFHxS (<0.04 μg kg^{-1}). In contrast to fish meals, were PFUnDA were one of the more dominant PFAS compounds, the salmon feeds contained low concentrations of PFUnDA (Table 4). As for fish meals, PFOS was also the dominant PFAS in fish feed, with concentrations of 0.06–2.37 μg kg^{-1}. Whereas for PFOA, which was only found at low levels in the fish meals (ND-0.09 μg kg), was present in higher concentrations in one of the aquafeeds (0.26 μg kg^{-1}). The long-chain PFAAs (PFDA and PFHxS), which were scarcely detected in fish meal, were detected in several of the aquafeeds, indicating other sources than fish meal for these PFAS. Surveillance of Greek aquafeeds detected and quantified PFOS and PFOA among 14 targeted PFAS [27], and similarly in two Danish aquafeeds PFOS and to a lesser degree PFOA were the quantified PFAS [22]. In the present study the four PFAS that have been risk assessed (PFOS, PFOA, PFNA, PFHxS) [12] were the main contributors (53–87 %) of the total PFAS load in salmon feed. As for food producing land animals [12,29], assessment of the feed-to-food transfer of these 4-EFSA PFAS based on PFAS specific kinetics [12,48] in farmed Atlantic salmon is needed to set appropriate ML in salmon feed to meet the set MLs in seafood. The current MLs are limited to four PFAS based on several similar effects in animals, toxicokinetics and observed levels in human blood [12]. However, minor PFAS observed in salmon feeds in the present study such as PFUnDA, PFDoDA and PFTrDA, are known to be involved in developmental and hepatic toxicity [12], and the level of PFUnDA in human blood, although lower than the four EFSA-PFAS, is relatively increasing over time as opposed to most EFSA-PFAS. This warrants the need for extended risk assessment beyond the four EFSA-PFAS including assessment of feed-to-salmon transfer to set appropriate feed ML in order to protect consumers.

Interestingly, the levels of the six precursors were much higher than the well-known six targeted PFCA/PFSA. Fig. 1 shows the concentrations of precursors in combination with the PFAS in relation to each other. Especially the fluorotelomer sulfonates (FTS), including 6:2FTS, 4:2FTS, 8:2FTS, and 10:2FTS, were detected at higher concentrations, with 6:2FTS at highest levels fish feed number 2 (FF2) (1.56 ± 0.11 μg kg^{-1} ww), followed by FF1 (1.45 ± 0.02 μg kg^{-1} ww). Similarly, 4:2 fluorotelomer sulfonate (4:2FTS) was most abundant in FF2 (3.81 ± 1.37 μg kg^{-1} ww), followed by FF1 (1.23 ± 0.14 μg kg^{-1} ww). Also, 8:2 fluorotelomer sulfonate (8:2FTS) and 10:2 fluorotelomer sulfonate (10:2FTS) were detected at notable levels in FF1 (1.45 ± 0.02 μg kg^{-1} ww and 1.3407 ± 0.0031 μg kg^{-1} ww, respectively), further reinforcing the predominance of these precursors. Additionally, a more well known PFOS precursor, perfluorooctane sulfonamide (FOSA), a known precursor to PFOS, was detected at elevated concentrations in FF2 (1.69 ± 0.49 μg kg^{-1} ww) and FF1 (1.38 ± 0.14 μg kg^{-1} ww).These findings highlight the prevalence of PFAS precursors of the persistent perfluoroalkyl acids (PFAAs), in salmon feed, raising concerns about their potential transformation into more persistent PFAS, which could contribute to exceedances of EU food safety limits for PFOS, PFOA, PFNA, PFHxS, ΣPFAS4. In chicken, the PFAS precursors Me-and EtFOSAA, FOSAA and FOSA can be biotransformed into PFOS when chickens are fed contaminated hay or barely, and their presence would contribute to an underestimation of the feed-to-food transfer of PFOS [29]. For fish, studies on rainbow trout also describe the biotransformation of PFOA from a precursor (8:2 fluorotelomer carboxylic acid)[30], and there is difference in PFAS biotransformation pathways among species [49]. In the present study, the level of precursors in the feed ingredients was lower than those observed in three of the four feed samples. On feed sample had a no detectable precursors at all, indicating a possible large variation of the source for “masked” PFAS in salmon feeds. Although this study included a wide range of feed types, the selection and analyses may not reflect the variety that could be present in commercial salmon feed. In addition to the source of feed ingredients other sources could include packaging materials [50], and possibly other feed production and processing steps, could be co-sources for precursors in salmon feeds due to the wide industrial application of PFAS. The contribution of these precursors to the overall feed-to-food transfer during a production cycle for Atlantic salmon remains to be investigated, as their potential transformation into more persistent PFAS could contribute to exceedances of EU food safety limits for PFOS, PFOA, PFNA, PFHxS, and ΣPFAS4 As feed ingredients and feed

3.2. Suspect and nontarget screening

In the non-target screening of PFAS, application of a PFAS-specific workflow and data filtering resulted in the retention of 142 compounds. Among these, only 12 PFAS were identified with high confidence (Table S2), including nine confirmed using reference standards (Level 1: Confirmed by Reference Standard) and three classified as Level 2. Molecular networking (Figures S2) and Kendrick mass defect Fig. 2 analyses enabled the characterization of four homologous series, revealing structural relationships among the detected PFAS. These included two perfluoroalkyl sulfonates (PFHpS [C7] and PFOS [C8]), two perfluoroalkyl sulfonamides (FBSA [C4] and PFOSA [C8]), four fluorotelomer sulfonates (4:2 FTS, 6:2 FTS, 8:2 FTS, and 10:2 FTS), and two N-substituted perfluoroalkyl sulfonamides (N-MeFOSAA and N-

Table 4
Concentrations μg kg^{-1} and limit of quantifications (LOQ) of PFAS in four randomly selected fish oil, plant meals and plant oils used in Norwegian Atlantic salmon feed production. Concentrations for this study are given as “lower bound” (LB) with values <LOQ set equal to zero. ND= not detected. Abbreviations for PFAS are given in the main text, the four PFAS currently risk assessed for human consumption by EFSA are given in bold, the contribution of these four-EFSA PFAS to total PFAS is given as percentage.

			PFOA	**PFNA**	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
	production	*type*							
fish feed 1	Norway	starter	0.26	0.65	0.15	0.17	0.035	0.075	0.02
fish feed 2	Norway	starter	ND	0.025	0.005	0.045	0.01	0.015	ND
fish feed 3	Norway	grower	ND	0.025	0.01	0.04	ND	ND	ND
fish feed 4	Norway	grower	ND	0.08	0.01	0.025	0.01	ND	0.005

			PFBS	**PFHxS**	PFHpS	**PFOS**	total LB	% 4-EFSA of total
	production	*type*						
fish feed 1	Norway	starter	ND	0.075	0.015	2.365	3.82	86 %
fish feed 2	Norway	starter	ND	ND	ND	0.06	0.16	53 %
fish feed 3	Norway	grower	ND	ND	ND	0.235	0.31	84 %
fish feed 4	Norway	grower	ND	ND	ND	0.275	0.41	87 %

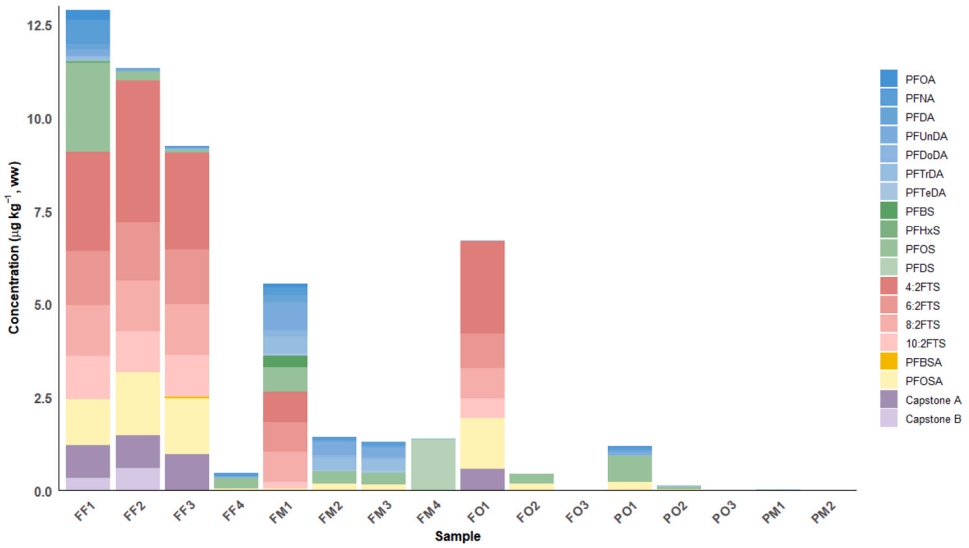

Fig. 1. Concentrations (μg kg^{-1} wet weight) of PFAS classes in randomly selected feed and feed ingredient samples, including fish feed (FF), fish oil (FO), commercial fish meal (FM) utilized in Norwegian salmon feed production, plant oil (PO), and plant meal (PM).

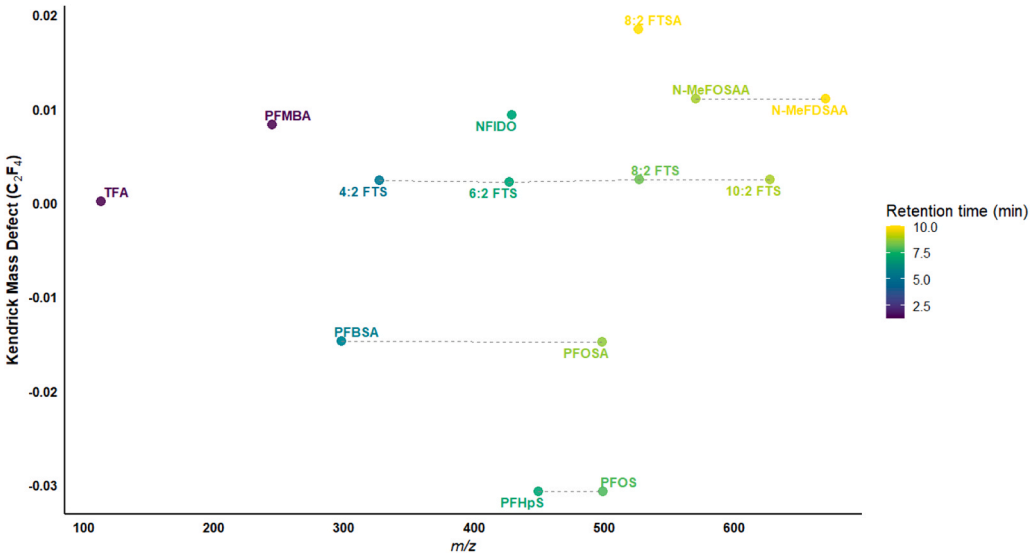

Fig. 2. CF2 Kendrick mass defect (KMD) obtained from Compound Discoverer for PFAS identified through nontarget screening. Four homologous series with repeating CF2 unit are depicted. The KMD for C_2F_4 repeating units is plotted against the mass-to-charge ratio (m/z). Four selected homologous series (HS) are shown. Each feature is represented by a circle, with color indicating retention time, illustrating chromatographic trends within each series.

MeFDSAA). In addition to confirming the presence of well-known PFAS such as PFHpS and PFOS and precursors such as FBSA and FOSA, also found in feed ingredients by others [21,22,27], the wide-scope screening identified the presence of four precursors which have not before been reported in fish meals and other aquafeed ingredients. The presence of the eight precursor compounds (i.e., FBSA, FOSA, N-MeFOSAA, N-MeFDSAA, 4:2 FTS, 6:2 FTS, 8:2 FTS, and 10:2 FTS) is particularly significant due to their known transformation pathways into more persistent PFAS, including PFSAs and PFCAs [51,52]. These precursors contribute to the long-term environmental burden of PFAS and may serve as indirect sources of contamination in aquatic ecosystems and aquafeed matrices. A target analysis revealed substantial amounts of the PFOS precursors *N*-methyl and *N*-ethyl perfluorooctane sulfonamidoacetic acid (Me- and EtFOSAA), perfluorooctane sulfonamidoacetic acid (FOSAA), and perfluorooctane sulfonamide (PFOSA) in hay and barley grown on PFAS contaminated soil that was used as feed to farmed land animals [28,29]. Currently, there is a lack of information regarding the biotransformation of the fluorotelomer sulfonates identified in this study. [30,49] Surveillance studies have detected precursors such as FOSA and PFBS in fish meal [27]. In contrast, for laboratory animal (zebra fish, mice etc.) feeds, no meaningful levels of PFAS precursor were found [25], and in laboratory feed to fathead minnow (fish flakes, shrimp and blood worms) only precursors such as 6:2 FTS and FOSA were detected at low levels, and none in commercial aquafeeds used in fathead minnow farms [26].

3.3. TOP assay

The TOP assay was employed to verify the presence of targeted PFAS precursors detected through targeted analysis and to reveal and quantify additional overlooked oxidizable precursors. All fish oil, plant oil and plant meal samples showed no changes in PFCA concentrations, indicating the absence of oxidizable precursors. Fish meal samples only showed an increase in PFHpA at levels close to the limit of quantification

(LOQ). In contrast, fish feed samples, particularly, exhibited significant precursor activity, with observed increases in PFCAs after applying the TOP assay, alongside substantial reductions in fluorotelomer sulfonates. The analysis of PFCA concentrations across feed samples FF1 to FF3 revealed varying levels of change post-treatment. Sample FF2 showed increases of 0.29 μg kg^{-1} for PFHxA, 0.33 μg kg^{-1} for PFHpA, and 0.88 μg kg^{-1} for PFOA, while sample FF1 exhibited slightly higher increases: 0.32 μg kg^{-1} for PFHxA, 0.33 μg kg^{-1} for PFHpA, and 1.00 μg kg^{-1} for PFOA. Sample FF3 displayed the smallest increases, with 0.12 μg kg^{-1} for PFHxA, 0.17 μg kg^{-1} for PFHpA, and 0.52 μg kg^{-1} for PFOA. Please note, sample FF4 showed no increase in PFCA concentrations, indicating the absence of precursor activity in this sample, which in agreement with the targeted analysis results (Fig. 1). Overall, PFOA experienced the most significant increase across those samples with detectable changes. As discussed above, these samples showed high concentrations of fluorotelomer sulfonates. Whereas the oxidation of 6:2 FTS and 8:2FTS might lead to the formation of PFHxA and PFOA, respectively, the increase of PFHpA might be attributable to the presence of yet unidentified precursors. Given the concentration of fluorotelomer sulfonates found, PFHxA increases across all samples were below the expected rise in PFCA concentrations assuming that the precursors (fluorotelomer sulfonates) are completely converted into their corresponding PFCAs under ideal conditions. PFOA reached theoretical levels in sample FF1, with sample FF2 close, indicating effective conversion compared to the lower increase in sample FF3 and no change in sample FF4. However, PFOA is the terminal product of other identified precursor PFOSA. These findings suggest varying precursor abundance across the samples. The assay not only confirmed the presence of precursors but also demonstrated their potential to form environmentally persistent and bioaccumulative PFAS.

4. Conclusion

Herein a comprehensive assessment of PFAS occurrence in FM and key feed ingredients used in Norwegian Atlantic salmon feed production was conducted. Although the proportion of FM in salmon feeds has significantly decreased over the past two decades due to the shift towards plant-based ingredients, it still contributes to the presence of persistent PFAS in fish feeds. The considerable variability in PFAS levels among different FMs underscores the need for further research into geographical, seasonal, and species-specific differences in PFAS bioaccumulation within marine food webs and fish meal produced thereof. This will aid the possibility to both source fish meals with natural lower levels of PFAS and set appropriate ML for PFAS in fish meal in order to preventively protect consumer safety of Atlantic salmon farmed on these fish meals. Targeted PFAS analysis of other feed ingredients, including fish oil and plant-based materials, revealed lower but detectable levels of several PFAS. Some plant oils contained PFOS at concentrations exceeding those found in FM, suggesting an additional, so far underestimated source of PFAS in salmon feeds, which is likely to occur when crops are grown of PFAS contaminated soil. These results emphasize the need for comprehensive surveillance of all feed materials to fully document and assess the risks related to PFAS in aquaculture. In addition to fish meal, ML for PFAS in plant-ingredients should be established in order avoiding the entering of PFAS contaminated process plant-products in the salmon food production chain. The detection of various PFAS precursors, including fluorotelomer sulfonates and perfluoroalkyl sulfonamides, raises concerns regarding their potential transformation into more persistent and bioaccumulative PFAS compounds during feed-to-food transfer processes. Traditional surveillance of PFAS in salmon feeds is likely to underestimate the relevance of PFAS feed contamination to PFAS levels in farmed salmon, as they do not include PFAS-precursors and their transformation into legacy PFAS in farmed animals [28]. Future efforts should focus on identifying contamination sources of these PFAS precursors, and investigate their transformation pathways into known PFAS in farmed fish.

Environmental Implication

Per- and polyfluoroalkyl substances (PFAS) are synthetic organic environmental contaminants often referred to as “forever chemicals” that encompassing more than 7 million compounds. The long-chain perfluoroalkyl acids, have been found to bioaccumulate in the protein-rich tissues of marine organisms, including fish. Fishmeal produced from wild marine fish stocks is considered as one of the main sources for known PFAS in food for farmed animals. In this study, non-targeted wide scope screening identified “masked” PFAS precursors at higher concentrations than the legacy PFAS i, suggesting their relevance as potential indirect sources of persistent PFAS, which was confirmed with TOP assay.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marc Berntssen reports financial support was provided by Nation Research Council of Norway. Marc Berntssen reports financial support was provided by FHF. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.139078.

Data availability

Data will be made available on request.

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