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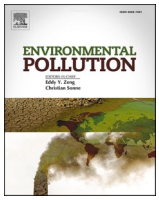

Assessment of PFAS pollution in fish and water from the United Kingdom and Spain and implications for human exposure☆

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are widespread environmental contaminants with significant persistence, bioaccumulation potential and human health concerns. This study assesses the occurrence, distribution and dietary exposure risks of PFAS contamination in edible fish from the United Kingdom and Spain. Fish samples (UK: n = 238; Spain: n = 50) and UK water samples (freshwater: n = 8099; groundwater: n = 2047 and saline water: n = 180) were collected between 2020 and 2024 and analysed for multiple PFAS compounds.

Results indicate widespread PFAS contamination in both fish and water, with PFOS being the predominant compound detected. However, significant geographical differences were observed. Spanish fish exhibited higher concentrations of long-chain PFAS (e.g., PFNA, PFDoDA, PFUnA) and emerging compounds (6:2 FTS), while UK fish showed elevated PFOS levels. Dietary exposure estimates revealed that intake from consumption of fish likely exceeds the EFSA tolerable weekly intake (4.4 ng/kg body weight) in both countries, with Spanish consumers facing a higher estimated intake (24.62 ng/kg) compared to British consumers (10.71 ng/kg).

PFAS contamination in UK water sources was extensive, with persistent pollution in both freshwater and groundwater. A hotspot in Moreton-in-Marsh was identified where contamination levels exceeded drinking water safety thresholds, likely linked to firefighting foam and industrial activities. The findings underscore the urgent need for stronger regulatory measures, continued biomonitoring, and remediation efforts to mitigate PFAS exposure risks.

1. Introduction

Environmental monitoring of per- and polyfluoroalkyl substances (PFAS) is essential for assessing human and ecological risks. Collaboration between academic institutions, governments and regulatory bodies is crucial to inform evidence-based decision-making and develop mitigation strategies where contamination hot-spots are identified.

PFAS, the terminal products commonly referred to as "forever chemicals," are a class of synthetic compounds with widespread applications, high environmental persistence, low acute toxicity and concerning long-term health effects. Used since the 1940s in industrial and consumer products, PFAS are characterized by alkyl-based structures containing the C_nF_{2n+1} functional group, where hydrogen atoms are fully replaced by fluorine or partially as in the case of fluorotelomers. The strong carbon-fluorine bonds provide exceptional water- and oil-repellent properties, making them valuable for industrial uses such as pesticides, electronics, firefighting foams, food packaging, non-stick cookware, stain-resistant textiles and water-resistant clothing. In 2018, the Organisation for Economic Co-operation and Development (OECD) and the United Nations Environment Programme (UNEP) identified 4730 distinct PFAS compounds in global use (OECD, 2018). Additionally, the U.S. Geological Survey (USGS) reported in 2023 that there are more than 12,000 types of PFAS, though not all can be detected with current testing methods (Smalling et al., 2023).

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The negative features of PFAS use including environmental persistence and toxicity in long term have raised significant environmental and public health concerns, prompting regulatory efforts worldwide. The U.S. Environmental Protection Agency (EPA) has established guidelines for specific PFAS compounds in drinking water and soil (U.S. Environmental Protection Agency, 2016), and in 2024 announced the first National Primary Drinking Water Regulation for six PFAS compounds, setting enforceable limits for PFOA, PFOS, PFNA, PFHxS, PFBS and GenX (U.S. Environmental Protection Agency, 2024). The Stockholm Convention classified perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) as Persistent Organic Pollutants in 2009 and 2019, respectively (UNEP, 2019), and perfluorohexane sulfonic acid (PFHxS) was added in 2022. In addition, long-chain perfluorocarboxylic acids (LC-PFCAs), their salts and related compounds, were added to Annex A of the Stockholm Convention in May 2025 (UNEP, 2025), expanding the global list of regulated PFAS and reinforcing international recognition of their persistence, bioaccumulation and toxicity. In the European Union, three substances (PFOS, PFOA, and PFHxS) and their related compounds are regulated under the POPs Regulation (EU) 2019/1021, which includes a few time-limited exemptions for specific uses. Moreover, the REACH regulation restricts long-chain PFAS for many applications (European Chemicals Agency, 2023). In response, manufacturers began substituting long-chain PFAS, such as PFOS and PFOA, with short-chain alternatives, such as perfluorohexanoic acid (PFHxA) and perfluorobutane sulfonate (PFBS), as well as novel compounds like HFPO-DA and Ammonium 4,8-dioxa-3H-perfluorononanoate (Munoz et al., 2019). The latter compounds differ from legacy PFAS homologues due to structural modifications that include one or more ether groups, which increases their mobility and, consequently, their environmental impact (Wang et al., 2019). Despite the use of alternatives to long-chain PFAS for several years, their toxicity and diffusion remains insufficiently studied (U.S. Environmental Protection Agency, 2021).

Unlike traditional persistent organic pollutants, which are generally lipophilic (Junqué et al., 2017), PFAS bioaccumulate in body tissues by binding to proteins, with an affinity for albumin in the blood, fatty acid-binding protein in the liver and organic anion transporter proteins in the kidneys (Ng and Hungerbühler, 2013; Martin et al., 2003; Shi et al., 2012). Their elimination is slow, leading to long-term bioaccumulation in organisms. A growing body of *in vitro* and *in vivo* toxicological research indicates that exposure to certain PFAS is associated with developmental, immune, neurotoxic, endocrine and carcinogenic effects in different model species (sensu Lagostena et al., 2024; Heintz et al., 2023; Paquette et al., 2023; Chowdhury, 2023; Dragon et al., 2023; Wasel et al., 2023; Adewuyi and Aga, 2022; Guo et al., 2022; Shane et al., 2020). Additionally, recent studies suggest that certain PFAS may act synergistically with microplastics, increasing their toxic potential (Soltanighias et al., 2024).

PFAS are widespread on a global scale (Tokranov et al., 2025), occurring in food (Pérez et al., 2014), drinking water (Gao et al., 2024) and air (Zhao et al., 2020) to cosmetic products (Namazkar et al., 2024) and even remote environments such as Arctic snow (Hartz et al., 2024) and dust aboard the International Space Station (Harrad et al., 2023). In humans, these persistent chemicals have been found in plasma (Coêlho et al., 2023), umbilical cord serum (Manzano-Salgado et al., 2015) and breast milk (Guerranti et al., 2013), indicating prenatal exposure due to placental transfer (Manzano-Salgado et al., 2015) and postnatal exposure via lactation (Guerranti et al., 2013).

The most important exposure pathways to PFAS in human populations are the ingestion of food and drinking water (Agency for Toxic Substances and Disease Registry [ATSDR], 2021; Sunderland et al., 2019; Domingo and Nadal, 2019), and dietary intake can account for over 90 % of the exposure (Stahl et al., 2011). Several studies have determined that PFAS levels in foodstuff usually follow a trend: fish and seafood, followed by meat, milk products and finally beverages, vegetables and fruits. Fish are particularly vulnerable to PFAS exposure due to their position within trophic systems and their direct contact with both dissolved and particulate pollutants (Brown et al., 2023). Furthermore, inhalation of PFAS in indoor and outdoor air and skin contact with PFAS-containing products should also be considered (Ragnarsdóttir et al., 2024). On top of that, the general population is exposed to low levels of PFAS from various sources such as food packaging and consumer products.

The United Kingdom has been at the forefront of PFAS monitoring, with the Environment Agency tracking PFAS in national waters since 2014 and in fish since 2020. Open access to the Environment Agency's monitoring data, such as that available through the Open WIMS data, offers valuable insights into contamination trends and presents a unique opportunity to address some of the knowledge and data gaps. In Spain, the Spanish National Research Council (CSIC) has led extensive research on PFAS occurrence, transport and environmental impact, while also advancing analytical methodologies (Campo et al., 2016; Pignotti et al., 2017; González-Gaya et al., 2019; Bautista et al., 2024). The establishment of the *National Office for Scientific Advice* (ONAC) for the executive branch and the *Office for Science and Technology of the Congress of Deputies* (Oficina C) for the legislative branch illustrates Spain's commitment to integrating scientific expertise into policymaking. These two countries thus represent contrasting regulatory and monitoring contexts—Spain operating under evolving EU directives and decentralized enforcement, and the UK adapting its national frameworks post-Brexit with a longer history of centralized monitoring.

In this study, we assessed the occurrence of 47 distinct PFAS in edible fish from the UK and Spain, analysing 288 fish samples from 17 commonly consumed species. We evaluated potential human health risks by estimating weekly intake levels based on the sum of 4 specific PFAS quantified in fish and comparing them with the European Food Safety Authority's Tolerable Weekly Intake thresholds. Additionally, a suspect screening approach was used to tentatively identify unknown PFAS in the fish samples. To contextualize PFAS contamination in the UK, we analysed Open WIMS data to examine spatial trends and compound profiles in freshwater, groundwater and saline water.

This study provides a comprehensive assessment of PFAS contamination in aquatic environments of the UK and Spain, and the consequent risks to consumers, integrating data from research institutions, government databases and scientific literature. The findings contribute to understanding PFAS distribution in fish and water and aim to inform future risk assessments, regulatory decisions and mitigation strategies.

2. Materials and methods

2.1. Fish samples

Three sampling campaigns were carried out during 2020, 2022 and 2024 to monitor the presence and concentrations of PFAS in fish muscle (n = 102) and whole fish (n = 136) from the United Kingdom and fish muscle from Spain (n = 50). Of these, 100 samples were collected and analysed by the University of Birmingham (UoB) and IDAEA-CSIC using the methods described in this paper. Data on PFAS concentrations in a further 188 UK fish samples were obtained from Environment Agency and Cefas monitoring programmes, with results made available through open-access sources Defra data services platform. These data were generated using different analytical methods from those used in our study.

The fish samples included seventeen species, all of them captured in the Atlantic Ocean and the Mediterranean Sea for marine fish or from inland waters for freshwater species. Atlantic fish from the UK were collected across multiple locations (Fig. 4), whereas Mediterranean fish were all collected at a single site in Spain (Fig. S3). These fish species were representative of local consumption, encompassing coley, dab, hake, lemon sole, mackerel, plaice, sardines, sea bass, mullet, trout, whiting, cod, flounder, herring, anchovies, roach and chub. Information on length, weight, date of sampling and catch location was recorded.

After collection, samples were wrapped in aluminium foil to avoid contamination and frozen at −23 °C until further analysis in the laboratory.

Two distinct sample sets were analysed: whole fish (provided by the Environment Agency) and muscle fillets (sourced from the University of Birmingham, CSIC-IDAEA and the Environment Agency). The whole fish were intended to assess total body burden, while the muscle fillets represented edible portions. This distinction is important, as internal organs such as the liver often accumulate higher PFAS concentrations than muscle tissue. Given the systematic differences between matrices, inferential statistics were conducted on fillet samples only, whereas whole-fish data are reported descriptively. The number of samples per species and country is summarised in Table S7, which also indicates the data source.

Fish samples were analysed at both research and regulatory laboratories (University of Birmingham–CSIC and Environment Agency, respectively). Detection limits varied across laboratories, ranging from 0.002 to 0.08 ng/g in research settings and 0.01–0.05 ng/g in the regulatory monitoring (Table S5).

2.2. Water samples

The Water Quality Data Archive (Open WIMS data) from the Environment Agency contains records dating back to 2000 for approximately 2000 sampling locations annually across the UK for different chemical measurements. This legacy service is currently being phased out and replaced by the Water Quality Explorer platform (https://environment.data.gov.uk/water-quality-beta), which will fully substitute the archive by December 2025.

For this study, we included monitoring data on PFAS (in μg/L) measured from 2021 to 2024 in freshwater (n = 8099, including rivers, ponds, lakes, and reservoirs), groundwater (n = 2047) and saline water (n = 180) (Table S2). The dataset comprised results from two analytical approaches: (i) targeted analysis of $PFOA^{-}$ conducted in 2021 and (ii) expanded analysis of a wider suite of PFAS compounds from June 2021 to 2024. A total of 17 areas and 44 subareas were sampled annually (Fig. S1). This timeframe was chosen because it marks the implementation of a fully quantitative and validated method for PFAS analysis (Environment Agency, 2021).

Detection limits for the PFAS analysed in water ranged between 0.00001 and 0.0015 μg/L depending on the compound and matrix (Table S6). Two analytical methods were applied by the Environment Agency: (i) a targeted approach quantifying $PFOA^{-}$ in early 2021, and (ii) an expanded multi-compound method implemented thereafter.

2.3. Chemicals and standards

Solvents for residue analysis including ultra-pure HPLC grade methanol and water (CHROMASOLV® Plus), ammonium hydroxide and sodium hydroxide were purchased from Sigma-Aldrich (Steinheim, Germany). The Oasis Weak Anion-eXchange (WAX) 6 cc cartridges, 150 mg sorbent per cartridge, 30 μm, were purchased from Waters Corporation (Milford, USA). A mixture of pure native standards of PFAS (PFAC-MXH, 1.2 mL in methanol) and a mixture of Mass-Labelled PFAS standard used as a surrogate internal standard (MPFAC-HIF-ES, 1.2 mL in methanol), were provided by Wellington Laboratories Inc. (Ontario, Canada).

The following 47 perfluoroalkyl compounds were included in the study: Perfluorobutanoic acid (PFBuA), Perfluoropentanoic acid (PFPeA), Perfluorohexanoic acid (PFHxA), Perfluoroheptanoic acid (PFHpA), Perfluorooctanoic acid and Perfluorooctanoate anion (PFOA and $PFOA^{-}$, respectively), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDcA), Perfluoroundecanoic acid (PFUnA), Perfluorododecanoic acid (PFDoDA), Perfluorotridecanoic acid (PFTrDA), Perfluorotetradecanoic acid (PFTeDA), Perfluorobutane sulfonic acid (PFBuS), Perfluoropentane sulfonic acid (PFPeS), Perfluorohexane sulfonic acid (PFHxS), Perfluoroheptane sulfonic acid (PFHpS), Perfluorooctane sulfonic acid (PFOS), Perfluorononane sulfonic acid (PFNS), Perfluorodecane sulfonic acid (PFDcS), Perfluorobutane sulfonamide (PFBSA), Perfluorohexane sulfonamide (PFHxSA), Perfluorooctane sulfonamide (PFOSA), 1H,1H,2H,2H-Perfluorohexanesulfonic acid (4:2FTS), 1H,1H,2H,2H-Perfluorooctanesulfonic acid (6:2FTS), 1H,1H,2H,2H-Perfluorodecanesulfonic acid (8:2FTS), 1H,1H,2H,2H-Perfluorododecanesulfonic acid (10:2 FTSA), Bis(perfluorohexyl)phosphinic Acid (6:6PFPi), (Heptadecafluorooctyl)(tridecafluorohexyl)-phosphinic (6:8PFPi), Bis(heptadecafluorooctyl) phosphinic acid (8:8PFPi), N-ethyl perfluorooctane sulfonamido acetic acid (NEtFOSAA), N-methyl perfluorooctane sulfonamido acetic acid (NMeFOSAA), 4,8-dioxa-3H-perfluorononanoic acid (ADONA), 2-Perfluorodecyl ethanoic acid (FDEA 10:2), 3-perfluoropropyl propanoic acid (FPrPA 3:3), 3-perfluoropentyl propanoic acid (FPePA 5:5), 3-perfluoroheptyl propanoic acid (FHpPA 7:3), 8-Chloroperfluoro-1-octane sulfonate (Cl-PFOS), 9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS), 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS), Perfluoro(2-ethoxyethane)sulfonate (PFEESA), Hexafluoropropylene oxide-dimer acid (HPFO-DA), Perfluoro(2-ethoxy-2-fluoroethoxy)- acetic acid, ammonium salt (EEA-NH4), Perfluoro-4-ethylcyclohexane sulfonate (PFECHS), Perfluorododecane sulfonic acid (PFDoS), Perfluoro-3-methoxypropanoic acid (PF3Prop), Trifluoroacetic acid (TFA) and Trifluoromethanesulfonic acid (TFSA).

2.4. Extraction and purification

The method for extraction and purification of PFAS from fish was adapted from a validated method described in Llorca et al. (2012). Briefly, each fish sample was first prepared by separating the edible muscle tissue from the whole fish using a clean stainless-steel scalpel. The tissue was then homogenized, and 2 g of the homogenate was transferred to a 50 mL polypropylene centrifuge tube for extraction. At this stage, 10 μL of the internal standard mix MPFAC-HIF-ES (prepared as a 1:2 dilution of the initial commercial concentration in methanol) was added, followed by 10 mL of methanol containing 10 mM NaOH. The mixtures were subjected to orbital shaking digestion for 2 h at 120 rpm and then centrifuged at 2000 rpm for 10 min at room temperature.

The supernatant (approximately 8 mL) was transferred to a 15 mL polypropylene centrifuge tube and evaporated under a gentle stream of nitrogen until the volume was reduced to approximately 2 mL. The extract was reconstituted with 50 mL of ultra-pure HPLC water and homogenized using a vortex mixer.

Extraction and purification were performed using solid-phase extraction (SPE) with weak anion exchange (WAX) Oasis 6 cc cartridges on a manifold. The cartridges were conditioned with 4 mL of HPLC-grade methanol, followed by 4 mL of HPLC-grade water under gravity flow. The samples were then loaded onto the cartridges for extraction and purification by gravity flow. After drying the cartridges under vacuum for 15 min, the PFAS compounds were eluted with 5 mL of 0.1 % ammonium hydroxide in methanol.

The eluates were evaporated to dryness under a gentle stream of nitrogen, transferred to liquid chromatography (LC) vials, evaporated again, and reconstituted with 100 μL of a 9:1 water:methanol solution. One procedural blank was included in each batch of samples. Samples were stored at 4 °C until instrumental analysis.

2.5. Instrumental analysis, data processing and quality assurance

2.5.1. Target analysis

A total of 100 fish samples were analysed by the UoB and IDAEA-CSIC laboratories, comprising 50 fish from Spain and 50 from the UK. These samples were processed and quantified in-house using the ultra-performance liquid chromatography (UPLC) coupled with a high-resolution mass spectrometer (HRMS), as described below. The remaining 188 fish samples, along with all water samples (n = 10,327,

including freshwater, groundwater and saline water), were analysed on behalf of the Environment Agency and the Department for Environment Food and Rural Affairs (DEFRA) at the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) laboratories using a fully quantitative and validated method for PFAS analysis (Environment Agency, 2021).

The quantification of PFAS in fish at UoB and IDAEA-CSIC was performed using UPLC (Acquity, Waters Corporation, Milford, MA, USA) coupled with a HRMS Q-Exactive (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source operating in negative ionization mode.

Chromatographic separation was achieved using a mixed-mode analytical column (SeQuant® ZIC®-HILIC column, 2.1 mm i.d., 100 mm length, 3.5 μm particle size) from Merck KGaA, Darmstadt, Germany. The column was maintained at 25 °C. The mobile phase consisted of 10 mM ammonium formate in water (A) and methanol (B). The gradient program began with 5 % B for 1 min, increased to 45 % over 4 min, further increased to 95 % over another 4 min, held for 2 min, and reconditioned at 5 % B for 3 min. The flow rate was set at 0.3 mL/min. This configuration enabled the simultaneous quantification of short-, medium- and long-chain PFAS with distinct physicochemical properties while maintaining high analytical performance across the carbon-chain spectrum.

The mass spectrometer parameters were as follows: gas (nitrogen) temperature, 350 °C; nebulizer pressure, 40 psi; sheath gas temperature, 350 °C; sheath gas flow, 10 L/min; capillary voltage, 3500 V; and nozzle voltage, 1000 V. The mass range for MS and MS/MS acquisition was 60–900 m/z. The Q-Exactive Orbitrap mass spectrometer operated in full scan acquisition and, in parallel, in data-independent acquisition (DIA) mode, with all-ion fragmentation (AIF) employed to ensure comprehensive ion fragmentation without selection bias.

Instrumental blanks consisting of 9:1 water:methanol were analysed every three sample injections to monitor system contamination. Calibration standards were run at various points during analysis—before, during, and after sample batches—to monitor sensitivity drifts and ensure data reliability. Data acquisition and interpretation were carried out using Xcalibur 4.0 software (Thermo Fisher Scientific, San Jose, CA, USA).

2.5.2. Suspect screening

Raw data obtained from the UPLC-HRMS Q-Exactive was processed using Compound Discoverer 3.3 SP2 (Thermo Fisher Scientific) following the workflow and the tentative criteria as described by Schymanski et al. (2014).

A comprehensive suspect screening strategy was applied using a compiled database that integrated multiple sources. This included: (i) a custom internal database developed from analytical standards analysed under our specific laboratory conditions; (ii) suspect lists and metadata from the NORMAN network (Bautista et al., 2024; Montes et al., 2022); and (iii) various online databases such as the Environmental and Food Safety (EFS) High Resolution Accurate Mass (HRAM) Compounds database, the PFAS NIST database (Place, 2021), ChemSpider for structural information and MzCloud for MS/MS spectral data. Note that MzCloud is a subscription-based platform and access to certain entries may be limited.

The initial automated processing generated over 70,000 candidate features in UK fish and over 80,000 in Spanish fish samples. Data were subsequently filtered using the following criteria: retention time alignment, detection of unknown features, grouping of compounds across all samples, elemental composition prediction, background subtraction (based on procedural blanks) and a minimum peak area of 1.0×10^6 a.u. (Schymanski et al., 2014; Montes et al., 2022). Suspects with an error mass of $< \pm 5$ ppm and detected in the three replicates were classified at confidence level 5. Features with molecular formula match scores $\geq$90 % were tentatively identified at confidence level 4. Further filtering steps included comparing isotopic patterns and annotations at level 4 with retention time matches (tolerance ±2.5 % min) and isotopic fit (>90 %), providing identification at confidence level 3. Tentative identification (level 2) was achieved by comparing the product ions from the MS/MS spectrum of a suspected compound with either the spectrum of a standard or a predicted fragmentation pattern available in online databases. Final confirmation (level 1) was obtained exclusively through comparison with a pure standard.

2.6. Dietary exposure estimates and threshold values

The concentration of PFAS in the edible portion of fish was assessed against the tolerable weekly intake (TWI) of 4.4 ng/kg body weight per week, as established by the European Food Safety Authority (EFSA, 2020) for the sum of four PFAS compounds: perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS).

The weekly intake of PFAS through fish consumption was estimated by multiplying the mean concentration of pollutants in fish (expressed in ng/g of fresh product) by the average weekly fish consumption rate (expressed in grams per week). The weekly consumption rates for adults were derived from estimates provided by the UK government, which are 280 g/week for adults, assuming an average body weight of 75 kg. While average fish consumption in Spain may be higher, a unified consumption rate was applied across both countries to allow direct comparison of PFAS exposure levels, isolating contaminant concentration as the primary variable of interest.

2.7. Statistical and data analysis

The statistical analyses were performed using R software 4.3.3 (R Core Team, 2024). PFAS concentrations in fish and water were expressed in ng/g and μg/L, respectively. When PFAS concentrations were below detection and quantification limits, values were substituted as follows: <LOD = ½ LOD and <LOQ = ½ (LOD + LOQ). Compounds quantified in less than 25 % of samples were excluded from statistical analyses. Mean and P50 (50th percentile, or median) were calculated to be able to directly compare the results to the EFSA threshold. The Kruskal-Wallis test was used to assess differences in pollutant concentrations by location. Species-level comparisons were restricted to muscle fillet datasets to avoid matrix-related bias (fillet vs whole fish). For each species and PFAS (including total PFAS (ΣPFAS)), differences between countries (UK vs Spain) were assessed using non-parametric Kruskal–Wallis and pairwise Wilcoxon rank-sum tests. Sample sizes by species and country are provided in Table S7. Pearson's correlation was applied to evaluate the relationship between fish weight, length and pollutant concentrations.

Data on PFAS concentrations at selected water sampling locations were extracted from the uncurated Open WIMS database provided by the Environment Agency. Given the limited associated metadata, data filtering focused on selecting reliable PFAS-location combinations monitored between 2021 and 2024. For each type of water (freshwater, groundwater and saline water) average concentrations with accompanying standard deviations were calculated for each individual PFAS, which were subsequently analysed in terms of compound profiles and spatial distributions.

2.8. Code and data availability

All code used for data processing, statistical analysis and figure generation in this study is openly available on GitHub at: EvaJunque/PFAS_Fish_Water_2020–24. The repository contains R scripts used to analyse PFAS concentrations, conduct statistical comparisons and generate all figures presented in this manuscript.

The analysis incorporates publicly available PFAS monitoring data from the Environment Agency's Water Quality Data Archive (Open WIMS). As the structure of the Open WIMS dataset can be complex to navigate, the repository includes detailed code and documentation

illustrating how to extract, clean and integrate relevant water quality data for PFAS. This aims to support other researchers and practitioners in reusing these valuable public datasets more easily.

The repository is structured to support reproducibility and includes.

- Raw and processed data (where permitted)
- Code for data wrangling and statistical analysis
- Scripts to generate all main and supplementary figures
- A README file with instructions for use

We encourage reuse of the code and welcome feedback or suggestions for improvement.

3. Results and discussion

3.1. Occurrence of PFAS in fish and water from the UK and Spain

The target analysis of PFAS in fish muscle fillets from Spain (n = 50, 2024) and the United Kingdom (n = 102, 2022–2024) revealed significant differences in concentration and composition across species and locations (Fig. 1; Table S1). In general, PFAS concentrations in fish from Spain were higher than those in the UK, likely due to their habitat in the Mediterranean Sea—a semi-enclosed water system—and their proximity to industrial sources. The most contaminated Spanish fish species were mackerel and dab, and UK species were coley and sea bass. In both countries, PFOS was the predominant compound detected, with mean concentrations of 4.34 ng/g in Spain and 2.14 ng/g in the UK (Table S1). The high frequency of PFOS detection (75 % in UK fillets, 100 % in whole UK fish, and 48 % in Spanish fish fillets) is consistent with its historical use and persistence in aquatic environments. PFNA and PFDcA were also frequently detected, with notably higher PFNA concentrations in edible portions of Spanish fish (mean = 2.06 ng/g) compared to UK fish (mean = 0.06 ng/g). Similarly, other long-chain congeners, such as PFUnA and PFDoDA, showed elevated levels in Spanish samples (mean = 0.85 ng/g and 1.27 ng/g, respectively). The presence of fluorotelomers such as 6:2 FTS was remarkable in Spanish fish (mean = 3.13 ng/g), whereas it was detected in only 21 % of UK fish muscle samples at much lower concentrations (mean = 0.07 ng/g; max = 0.55 ng/g). These UK results are broadly consistent with those reported by Androulakakis et al., (2022), who found 6:2 FTS in marine and freshwater fish from Northern Europe, including the UK, in the range of 0.05–0.36 ng/g and 0.05–0.23 ng/g, respectively. In contrast, the elevated concentrations observed in Spanish fish—reaching up to 18.85 ng/g—suggest more substantial local sources of 6:2 FTS.

Approximately 25 % of the British samples showed two ultra-short chain PFAS (Table S1; Fig. 1): TFA and TFSA. These findings are particularly significant given the growing concerns about the environmental persistence, mobility and potential health impacts of ultra-short-chain PFAS (Arp et al., 2024). However, the origin of TFA in the environment remains debated; it may stem from multiple sources, including the atmospheric degradation of certain perfluoroalkyl-containing substances such as hydrofluorocarbons (HFCs) and hydrochlorofluorocarbons (HCFCs), industrial discharges or degradation of fluorinated pharmaceuticals, pesticides and refrigerants (Freeling and

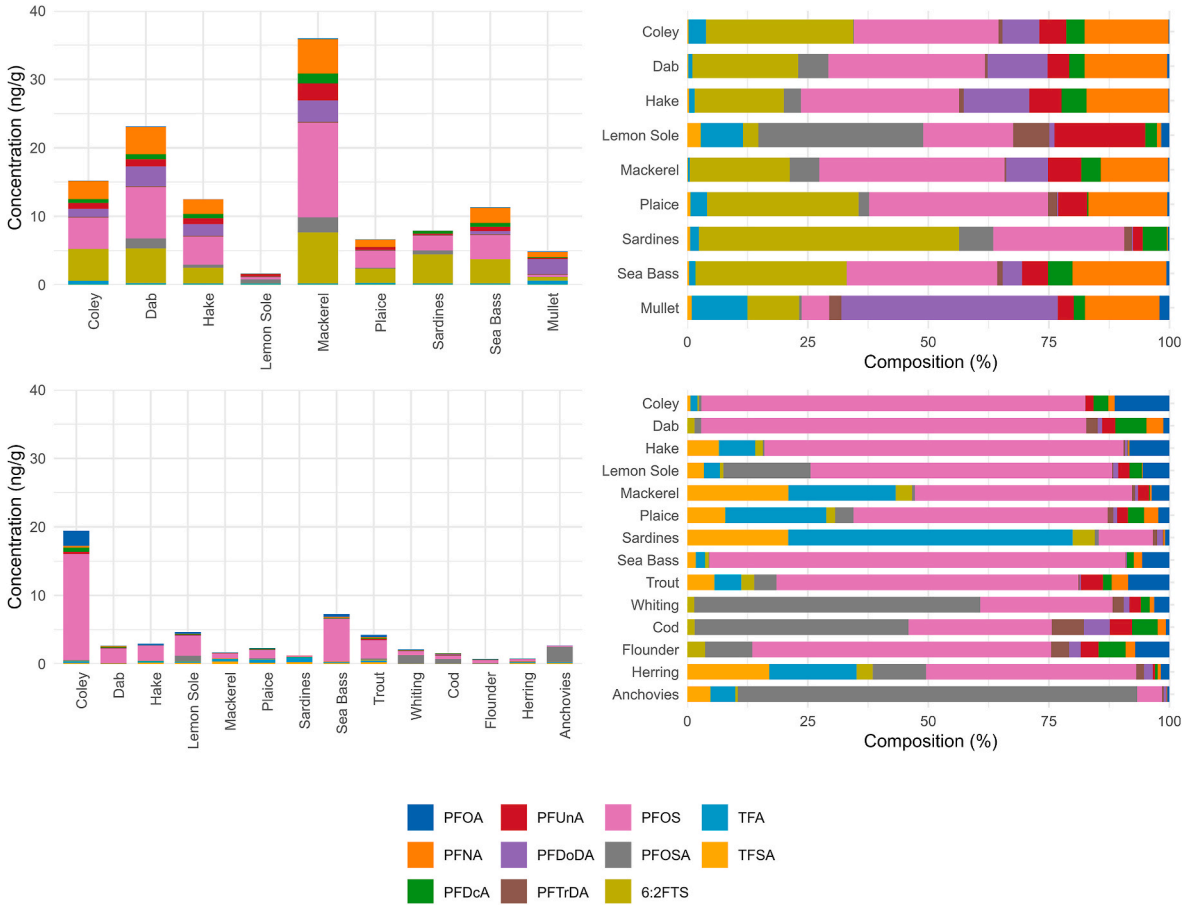

Fig. 1. Target analysis of PFAS in fish muscle fillet. Mean concentrations (in ng/g) and relative concentrations (in %) of the main PFAS compounds detected in muscle fillet from fish in Spain (top, n = 50, captured in 2024) and the United Kingdom (bottom, n = 102, captured during 2022–2024), grouped per fish species. Note that this plot only presents the compounds quantified in over 25 % of the samples in at least one country. Concentrations detected in fish from Spain were higher than those in the UK. The UK dataset combines targeted data from the UoB-CSIC and the Environment Agency.

Björnsdotter, 2023).

Additionally, both the UK and Spain have historically regulated PFAS under the EU REACH Regulation and although the UK has adopted its own version (UK REACH) post-Brexit, divergence from EU policy has so far remained limited.

Table S3 summarizes the results of the suspect PFAS screening in fish samples from Spain (n = 50) and the UK (n = 50) with a confidence level of 2. The suspect screening identified several additional PFAS compounds that were not included in the targeted analysis. In Spanish fish samples, 2-Perfluoropropyl-2-propanol, 6-(2,2,3,3,4,4,4-Heptafluorobutoxy)hexan-1-ol and Perfluoroalkyl Pentadecanedioic acid were detected in all samples, while TFA was found in 44 out of 50 samples. The most prominent peak in terms of signal intensity was observed for trifluoromethanesulfonic acid (TFSA). In UK fish muscle samples, TFSA was again among the most intense features and was detected in 35 out of 50 samples. Additionally, 6-(2,2,3,3,4,4,4-Heptafluorobutoxy)hexan-1-ol was found in 46 samples and N-dihydroxyethyl amino propyl-perfluorodecane amide in 38. Some unique compounds, such as 1,1,1,2,2,3,3,4,4-Nonafluorotetradecane, appeared in nearly all UK samples, highlighting the presence of novel or emerging PFAS. These results highlight the broader diversity of PFAS contamination in fish that may not be fully captured by standard targeted methods, reinforcing the importance of combining both analytical approaches to assess PFAS pollution comprehensively.

Water samples from the UK, collected between 2021 and 2024 (n = 8099 for freshwater; n = 2047 for groundwater; n = 180 for saline water), exhibited widespread PFAS contamination (Fig. 2, Table S2). PFOS was the most frequently detected compound in both freshwater and groundwater (99 % detection rate), with mean concentrations of 0.011 μg/L in freshwater and 0.015 μg/L in groundwater. These levels are concerning given PFOS's high bioaccumulation potential and regulatory restrictions on its use. PFOA anion was also widely present, with higher concentrations in groundwater (mean = 0.010 μg/L) than in freshwater (mean = 0.004 μg/L) and saline water (mean = 0.002 μg/L). Overall, PFAS were detected at a higher percentage in freshwater than in groundwater. The presence of short-chain PFAS, such as PFBuA and PFPeA, suggests potential ongoing emissions, as these compounds are increasingly used as substitutes for long-chain PFAS. Interestingly, 6:2 FTS was detected at relatively high concentrations in both freshwater (mean = 0.025 μg/L) and groundwater (mean = 0.029 μg/L), despite detected low concentrations in fish. This finding aligns with reports from the Environment Agency, which indicate that many members of the trade association for coatings, the Surface Engineering Association, have transitioned to using 6:2 FTS, with registrations under REACH indicating that it is being produced in the EU in the 10–100 tonnes per year band (Environment Agency, 2021).

Fig. 4 illustrates the spatial distribution of total PFAS (ΣPFAS) concentrations in fillet and whole fish across the United Kingdom. Comparison between surface and groundwater data (Figs. 5 and 6) reveals overlapping regions of elevated contamination, particularly in southern England, where both environmental and biological samples show increased PFAS levels. These co-occurrences suggest that local water contamination contributes to PFAS bioaccumulation in fish.

Among the compounds detected, PFOS was the most prevalent in both fish and water, indicating its continuing environmental persistence across UK aquatic systems. PFOA hotspots also coincide across matrices, further supporting the link between surface and groundwater contamination and PFAS uptake in aquatic biota. The combined use of research and regulatory monitoring data thus provides an integrated picture of PFAS occurrence and distribution in the UK.

The observed PFAS profiles in fish and water indicate possible accumulation patterns, with PFOS showing the highest concentrations in both matrices. Short-chain PFAS, such as PFBuA, PFPeA, and PFBuS, are more water-soluble and mobile, leading to their prevalence in aqueous

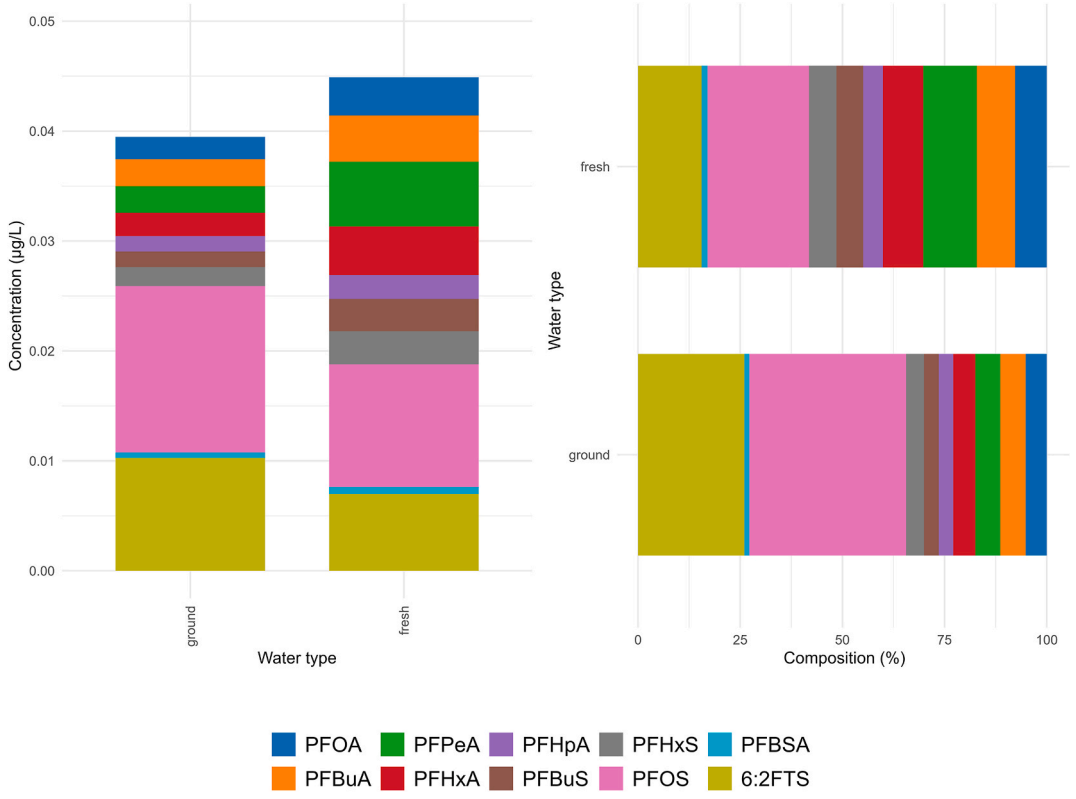

Fig. 2. Mean concentrations (in μg/L) and relative proportion (%) of the main PFAS compounds detected in freshwater (n = 8099) and groundwater (n = 2047) collected from 2021 to 2024 in the United Kingdom. Note that this plot only presents the compounds quantified in over 25 % of the samples. Data were sourced from the Environment Agency's national monitoring programme (Open WIMS data).

samples. Their higher solubility allows them to travel long distances, potentially exposing populations far from the original contamination sources (Li and MacDonald Gibson, 2022) and gradually accumulating in groundwater. Short-chain PFAS were introduced by industry as replacements for long-chain PFAS, such as PFOS, following regulatory restrictions. While they are generally considered less bioaccumulative and less harmful, their increased mobility has led to their widespread presence in the environment, highlighting concerns about the unintended consequences of this substitution. In contrast, long-chain PFAS, like PFNA, PFUnA and PFDoDA have a greater tendency to bioaccumulate in animal tissues, including fish, due to their strong affinity for proteins (U.S. Environmental Protection Agency, 2009) and are very rarely detected in water samples. Comparison of our data with fish and water samples from other countries reveals similar, though not identical, PFAS patterns, potentially reflecting variations in usage profiles (Pulster et al., 2022). Likewise, a recent nationwide study of freshwater fish fillets in the United States also found PFOS to be the dominant compound, accounting for most of the ΣPFAS burden across sites (Barbo et al., 2023).

3.2. *Species-specific differences between fish muscle fillet from Spain and the UK*

To further assess geographical variations in PFAS contamination, we

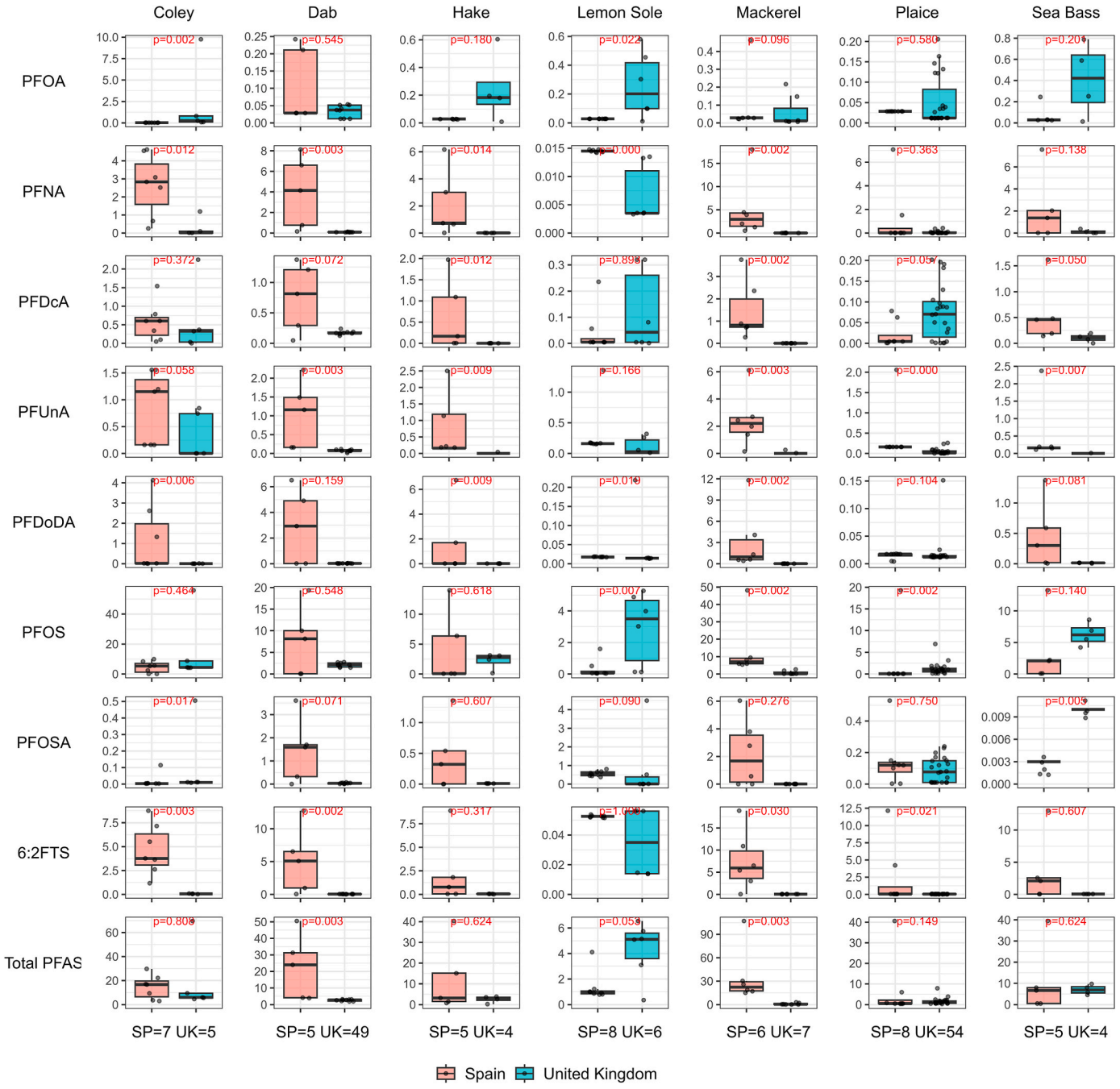

Fig. 3. Boxplots showing the differences in the total amounts (ng/g) of the main PFAS compounds quantified in fish muscle from the same fish species sourced from Spain (n = 50) and the United Kingdom (n = 102). Under each species, the sample size (N) is reported for Spain (SP) and the United Kingdom (UK), respectively. The UK dataset combines targeted data from the UoB-CSIC and the Environment Agency. *P-values* are from Kruskal-Wallis tests. *Asterisks (*)* indicate statistically significant differences ($p < 0.05$) between countries.

compared the PFAS concentrations in fish muscle fillet from the same species in Spain and the UK (Fig. 3). Statistically significant differences were observed for several compounds, as determined by the Kruskal-Wallis test (p-values shown in Fig. 3).

Overall, the most contaminated fish species from Spain were mackerel and dab, particularly for long-chain compounds such as PFOS and 6:2 FTS. In contrast, the most contaminated UK fish species were coley and sea bass, with PFOS as the predominant compound. In addition to long-chain PFAS, ultra-short-chain compounds such as TFA and TFSA were more frequently detected in UK fish, especially in sardines, mackerel, herring and plaice—species in which these compounds were rarely found in Spanish samples.

Spanish fish exhibited significantly higher concentrations of long-chain PFAS, including PFNA, PFUnA and PFDoDA, compared to their UK counterparts. This pattern may reflect differences in environmental exposure between regions, possibly influenced by historical or ongoing emissions in Spanish aquatic environments. Potential contributing factors could include industrial activity, wastewater discharges, or atmospheric inputs in the Mediterranean region. Notably, PFNA in Spanish fish reached mean concentrations exceeding 2 ng/g, whereas levels in UK fish remained below 0.2 ng/g. Additionally, the detection frequency and concentrations of 6:2 FTS were notably higher in Spanish fish, reinforcing the hypothesis that Spanish aquatic systems might be more affected by fluorotelomer-based contamination sources.

Conversely, PFOA concentrations were generally higher in UK fish (mean in UK fish = 0.21 ng/g). This finding aligns with the persistence of PFOA in UK freshwater and groundwater (Figs. 2 and 5), where it was detected in 70–99 % of samples (Table S2). The greater prevalence of PFOA in UK fish may reflect historical usage patterns or sediment-associated contamination, leading to prolonged exposure through trophic transfer. Fig. S2 illustrates the relationship between PFOA concentrations and fish length/weight, showing a general trend of increasing PFOA levels with larger fish specimens, as long-chain PFAS tend to accumulate in the food chain. The confidence interval suggests variability in accumulation, likely influenced by factors such as species-specific metabolism, habitat contamination and trophic position.

3.3. Dietary exposure assessment

The estimated dietary intake of Σ4 PFAS regulated by EFSA (PFHxS, PFOA, PFNA, and PFOS) was higher for Spanish consumers (mean = 24.62 ng/kg) than for British consumers (mean = 10.71 ng/kg; Table S4). This difference is primarily driven by the elevated PFOS and PFNA concentrations in Spanish fish, which were significantly higher than those in UK fish (Table S4, Table S1). Notably, the mean PFNA intake from Spanish fish (2.06 ng/kg) was nearly 35 times higher than from UK fish (0.06 ng/kg), suggesting a greater exposure risk in Spain.

The mean estimated intake from Spanish fish (24.62 ng/kg) and British fish (10.71 ng/kg) both exceed the EFSA tolerable weekly intake (TWI) of 4.4 ng/kg, indicating a potential health concern for frequent fish consumers. Even at the median exposure level (P50), Spanish consumers (3.04 ng/kg) are closer to the EFSA limit than British consumers (2.76 ng/kg). Given that EFSA's TWI is designed to be protective against long-term adverse health effects, these findings suggest a need for continued monitoring of dietary PFAS exposure and the effectiveness of TDIs as a protective measure. The variation between species suggests that people with preferences for specific fish such as coley and sea bass (both UK and Spain) and mackerel, dab and hake (Spain) are at much higher risk of exceeding the threshold (Fig. 1), offering insights into potential targeted measures to reduce exposures. Additionally, Fig. S2 highlights the importance of considering fish size when assessing dietary exposure risks.

The findings indicate that fish consumption is a relevant pathway for PFAS exposure, with regional differences in contamination leading to higher estimated intake in Spain than in the UK. Given the frequent exceedance of EFSA's TWI, revising dietary guidelines, improving risk communication and strengthening regulations on PFAS emissions and fish monitoring are crucial to safeguard public health.

3.4. Distribution of PFAS in water from the UK

Fig. 5 presents the spatial and temporal distribution of PFOA in freshwater and groundwater across the United Kingdom from 2021 to 2024, showing notable variability between water sources. A recent report by the Environment Agency (2024) provides further insight into temporal trends of PFOA concentrations in English freshwater. Their

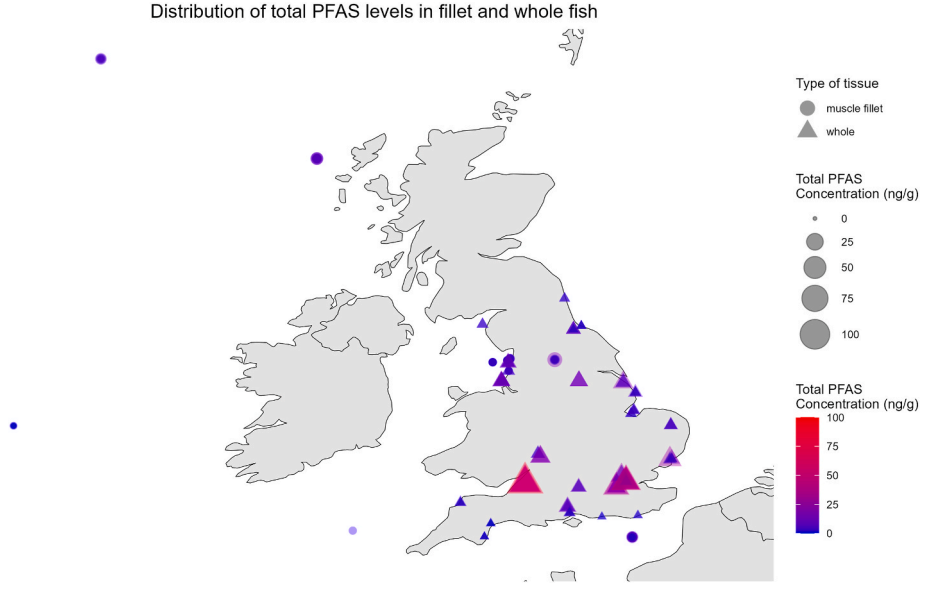

Fig. 4. Spatial distribution of total PFAS concentrations (ng/g) in fillet and whole fish collected across the United Kingdom between 2020 and 2024. Circles represent fish muscle fillets, while triangles indicate whole-fish samples. Symbol size and colour intensity correspond to increasing total PFAS concentrations. Data originate from the University of Birmingham–CSIC and Environment Agency monitoring campaigns. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

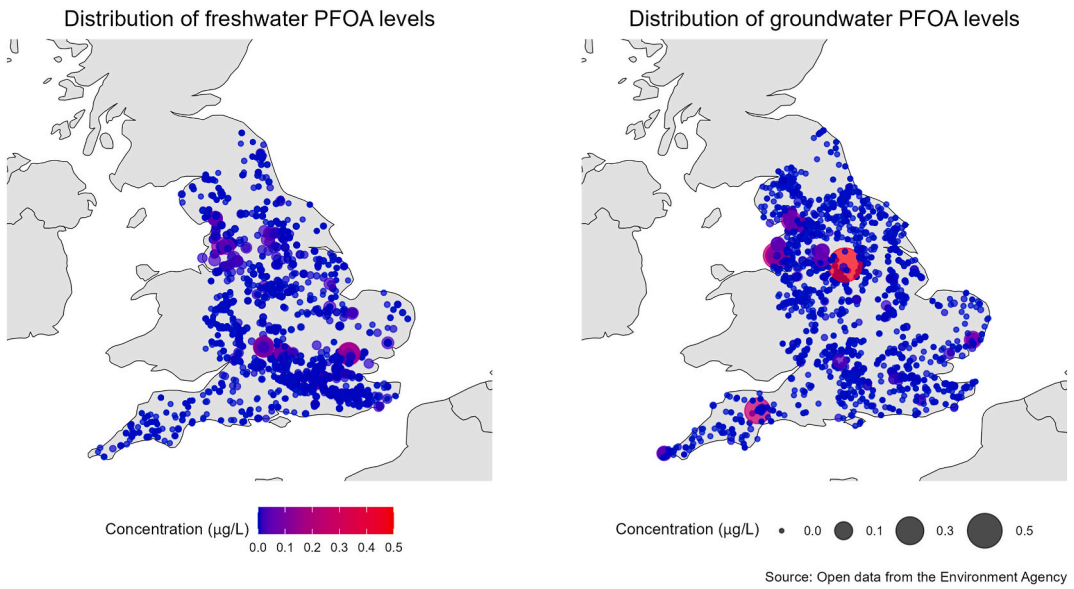

Fig. 5. Spatial distribution of PFOA concentrations (µg/L) in freshwater (left) and groundwater (right) across the United Kingdom between 2021 and 2024. Maps were created in R (ggplot2). Dot size represents the grouped PFOA concentration at each sampling location, while the colour gradient (blue to red) indicates increasing concentration levels. Data originate from the Environment Agency's national monitoring programme (Open WIMS data). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

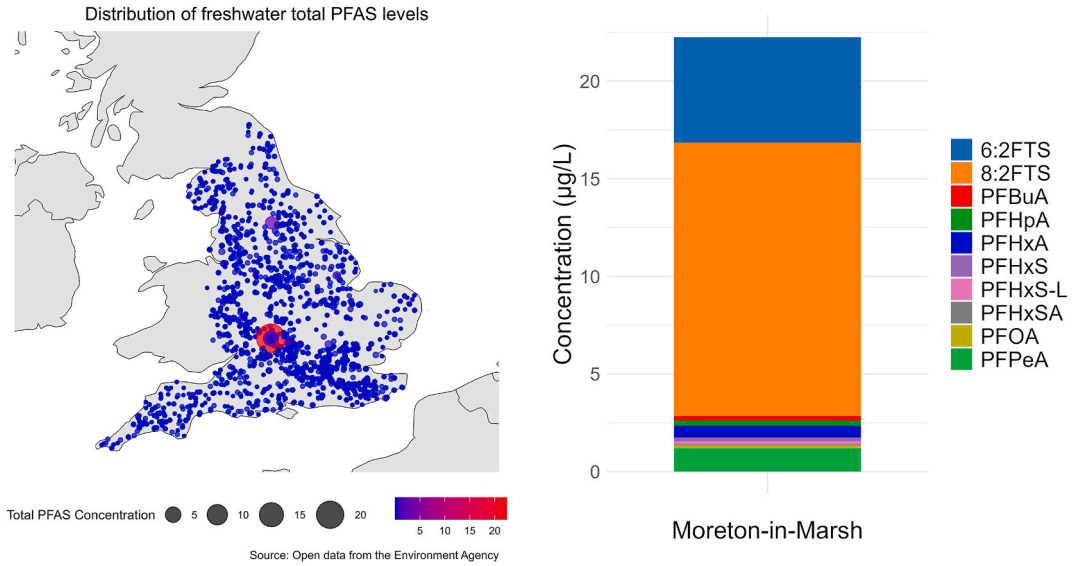

Fig. 6. Spatial distribution of total PFAS concentrations (µg/L) in freshwater across the United Kingdom between 2021 and 2024 (left), and compound-specific profile of the ten most abundant PFAS detected at the most contaminated site, Moreton-in-Marsh (right). Data originate from the Environment Agency's national monitoring programme (Open WIMS data).

monitoring data, collected between 2016 and 2022, suggest a gradual decline in PFOA concentrations over time, although the trend was less pronounced compared to that observed for PFOS. While PFOA remains widely detected across freshwater environments, these findings indicate that regulatory measures may be contributing to a slow reduction in environmental levels. Nevertheless, the persistence and mobility of PFOA highlight the need for continued monitoring and mitigation strategies to further address legacy contamination.

Fig. 6 illustrates the total PFAS burden in freshwater, showing a widespread presence of multiple PFAS compounds across the UK. The highest contamination levels were observed in Moreton-in-Marsh (22.43 µg/L), where mean concentrations exceeded regulatory thresholds for drinking water safety (100 ng/L for PFOA and PFOS; Water Supply (2016) (Water Quality),). In August 2024, the Drinking Water Inspectorate set a new stricter limit of 100 ng/L for the cumulative total of 48 different types of PFAS including several alternative compounds, which falls roughly in line with proposals from environmental organizations (Cleaning up UK drinking water | PFAS). It is important to note, however, that the samples assessed in this study are from raw, untreated environmental water sources, not final treated and blended drinking water. As such, comparisons with drinking water thresholds are indicative only and intended to provide context, rather than imply direct exceedance of regulatory limits for finished drinking water.

Fire-fighting training and fire stations along with military airfields and bases, wastewater treatment works and landfills have been identified as major sources of PFAS contamination, posing the highest potential risk for environmental pollution (Rotander et al., 2015). Moreton-in-Marsh has been identified as a significant freshwater PFAS

contamination site, primarily due to the historical use of firefighting foams at local training facilities, such as the Fire Service College. PFAS is an integral component of aqueous film forming foams (AFFF), a fire suppressant which have been manufactured and used for firefighting purposes since late 1960s (3M, 2022). The Environment Agency has acknowledged the legacy burden of such contamination and the complexity of remediating these high-risk sites. According to the Agency's commissioned report, the potential cost of remediating PFAS contamination in England ranges from approximately £31 billion to £121 billion, depending on the scope of remediation and the number of high-risk sites targeted, which could number between 2900 and 10,200 (Environment Agency, 2023).

4. Conclusions

This study provides a comprehensive overview of PFAS contamination in fish from the United Kingdom and Spain, and in UK water sources, integrating data from research and regulatory monitoring programmes. The findings reveal widespread PFAS occurrence, with notable regional differences that highlight the need for continued surveillance of both legacy and emerging compounds.

Beyond quantifying concentrations, our results underscore the importance of linking environmental and biological monitoring to better understand PFAS transport and bioaccumulation in aquatic food webs. The identification of hotspots associated with firefighting foam contamination and the exceedance of EFSA dietary thresholds point to the urgency of mitigating ongoing emissions through strengthening and enforcement of regulatory limits.

These insights are particularly relevant for the design of future monitoring frameworks under UK and EU chemical regulations. Coordinated approaches that harmonise detection methods, reporting standards and data integration between research and government agencies will be critical to improve comparability and maximise policy relevance.

Finally, the study demonstrates the value of open data and cross-sector collaboration in addressing complex contaminants such as PFAS, supporting evidence-based decision-making to protect both environmental and human health.

CRediT authorship contribution statement

Eva Junqué: Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Marta Llorca:** Writing – review & editing, Methodology, Funding acquisition. **Arianna Bautista:** Methodology. **Jon Barber:** Methodology. **Francesco Dondero:** Writing – review & editing, Funding acquisition. **Marinella Farré:** Writing – review & editing, Funding acquisition, Conceptualization. **Iseult Lynch:** Writing – review & editing, Funding acquisition, Conceptualization.

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.envpol.2025.127515.

Data availability

All code used for data processing, statistical analysis and figure generation in this study is openly available on GitHub at: EvaJunque/PFAS_Fish_Water_2020-24.

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