Environmental Pollution 339 (2023) 122738

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

# Biomagnification and temporal trends (1990–2021) of perfluoroalkyl substances in striped dolphins (*Stenella coeruleoalba*) from the NW Mediterranean sea☆

Odei Garcia-Garin[a,*], Asunción Borrell[a], Pere Colomer-Vidal[b], Morgana Vighi[a], Núria Trilla-Prieto[c], Alex Aguilar[a], Manel Gazo[a], Begoña Jiménez[b]

[a] *Department of Evolutionary Biology, Ecology and Environmental Sciences, and Biodiversity Research Institute (IRBio). Faculty of Biology. Universitat de Barcelona, 08028, Barcelona, Spain*
[b] *Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, IQOG-CSIC, 28006, Madrid, Spain*
[c] *Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, IDAEA-CSIC, 08034, Barcelona, Catalunya, Spain*

## ARTICLE INFO

*Keywords:*
PFAS
PFOA
PFOS
POPs
Cetaceans
Pollution

## ABSTRACT

Poly- and Perfluoroalkyl Substances (PFAS) are a well-known class of pollutants which can bioaccumulate and biomagnify with a vast majority being highly persistent. This study aims to determine the biomagnification rates of PFAS in sexually mature striped dolphins and to assess temporal trends on PFAS concentrations over the past three decades (1990–2021) in the North-Western Mediterranean Sea. Thirteen and 17 of the 19 targeted PFAS were detected in the samples of the dolphins' digestive content and liver, respectively, at concentrations ranging between 43 and 1609 ng/g wet weight, and 254 and 7010 ng/g wet weight, respectively. The most abundant compounds in both types of samples were linear perfluorooctanesulfonic acid (n-PFOS) and perfluorooctanesulfonamide (FOSA), which were present in all samples, followed by perfluoroundecanoic acid (PFUnDA), perfluorotridecanoic acid (PFTrDA) and perfluorononanoic acid (PFNA). Long-chain PFAS (*i.e.*, PFCAs C $\geq$ 7 and PFSAs C $\geq$ 6) biomagnified to a greater extent than short-chain PFAS, suggesting a potential effect on the health of striped dolphins. Environmental Quality Standards concentrations set in 2014 by the European Union were exceeded in half of the samples of digestive content, suggesting that polluted prey may pose potential health risks for striped dolphins. Concentrations of most long-chain PFAS increased from 1990 to 2004–2009, then stabilized during 2014–2021, possibly following country regulations and industrial initiatives. The current study highlights the persistent presence of banned PFAS and may contribute to future ecological risk assessments and the design of management strategies to mitigate PFAS pollution in marine ecosystems.

## 1. Introduction

Poly- and Perfluoroalkyl Substances (PFAS) are a large group of chemicals known for their persistence, ability to bioaccumulate in living organisms, and long-range environmental transport (Evich et al., 2022; Kannan, 2011; Khan et al., 2023). PFAS are used in various industrial and household applications, including surfactants, flame retardants, and food packaging (Giesy and Kannan, 2002; Kannan, 2011). Perfluorocarboxylates (PFCAs), such as perfluorooctanoic acid (PFOA), and perfluoro alkane sulfonic acids (PFSAs), such as linear perfluorooctane sulfonic acid (n-PFOS), are the most studied groups of PFAS (Buck et al., 2011; Li et al., 2020; Podder et al., 2021).

These chemicals have been extensively produced and used since the 1960s (Buck et al., 2011; Giesy and Kannan, 2001). Recently, they have been classified as global environmental pollutants (Kannan, 2011); PFOS and PFOA have been added to the list of the Stockholm Convention (SC) for global restriction and elimination in 2009 and 2019, respectively. Perfluorohexane sulfonic acid (PFHxS) was added in the Annex A of the SC and long-chain PFCAs were proposed for listing under the SC in 2022 (Stockholm Convention, 2022a; 2022b). Long-chain PFAS (PFCAs C $\geq$ 7 and PFSAs C $\geq$ 6) are being progressively substituted by short-chain PFAS (Stockholm Convention, 2022b; UNEP, 2009).

---

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author.

*E-mail address:* odei.garcia@ub.edu (O. Garcia-Garin).

https://doi.org/10.1016/j.envpol.2023.122738
Received 30 June 2023; Received in revised form 22 September 2023; Accepted 12 October 2023

PFAS constitute a planetary boundary issue according to the criteria established by MacLeod et al. (2014), which include (i) global diffuse pollution, (ii) newly discovered effects following the global spread of pollutants, and (iii) irreversible or poorly reversible effects. As with many chemicals in use, the vast number of PFAS in the class precludes a comprehensive assessment of their planetary boundary threat due to insufficient information. Cousins et al. (2022) tested this hypothesis by comparing the levels of PFOS, PFOA, PFHxS and PFNA in rainwater, soils, and surface waters, and concluded that the planetary boundary has been exceeded.

PFAS may cause adverse effects such as liver and thyroid dysfunction (Kudo et al., 2006), reproductive (Lau et al., 2003) and developmental toxicity (Berthiaume and Wallace, 2002), and immunotoxicity (DeWitt et al., 2012; UNEP, 2022, 2019, 2017, 2006). For instance, in sub-chronic low concentration doses, n-PFOS has been shown to reduce the viability of the F1 generation in rats, causing pup deaths shortly after birth (UNEP, 2006).

PFAS have been found in most trophic levels of marine food webs, including invertebrates (*e.g.*, Gómez et al., 2011; Haug et al., 2010), fish (*e.g.*, Haug et al., 2010), seabirds (*e.g.*, Colomer-Vidal et al., 2022; Escoruela et al., 2018), and marine mammals (*e.g.*, Ishibashi et al., 2008; Kannan et al., 2006, 2002, 2001; Lam et al., 2016; Schiavone et al., 2009; van de Vijver et al., 2003). PFAS can bioaccumulate and biomagnify (Bossi et al., 2005; Kannan et al., 2005; Khan et al., 2023; Martin et al., 2004), although short-chain PFAS may have a lower potential for bioaccumulation and biomagnification compared to long-chain PFAS (Goeritz et al., 2013; Renner, 2006).

Cetaceans, such as dolphins, are effective indicators of chemical pollutants due to their high trophic level, long lifespan, wide home range, and a variety of foraging habits. They may provide a comprehensive picture of PFAS exposure to living organisms, trends, and impact on marine ecosystems (*e.g.*, Andvik et al., 2023; Dorneles et al., 2008; Gui et al., 2019; Hart et al., 2008; Quinete et al., 2009; Stockin et al., 2021).

Most n-PFOS and PFOA time trend studies to date have been limited to short periods (10–15 years). The environmental fate of PFOS replacements such as PFHxS and perfluorobutane sulfonic acid (PFBS) remains poorly understood. Additionally, there is a lack of comprehensive information on the status and trends of the banned PFAS and their replacements in marine environments.

The aims of the current study were to determine the biomagnification rates of PFAS in sexually mature striped dolphins (*Stenella coeruleoalba*) from the NW Mediterranean Sea and assess temporal trends of PFAS concentrations over the past three decades (1990–2021). For this latter aim, we focused on sexually mature males to minimize intrapopulation variability, assuming that their diet and metabolism are relatively consistent, as they are not affected by the potential variation in nutritional requirements caused by growth and reproductive transfer to offspring, two factors that are known to influence the accumulation rate of many pollutants in mammals.

## 2. Material and methods

### 2.1. Sampling

Liver samples were collected from 48 sexually mature males of striped dolphin that stranded along the Spanish Catalan coast in the NW Mediterranean Sea (Fig. 1) during three separate periods between 1990 and 2021: 1990 (n = 19), 2004–2009 (n = 13), and 2014–2021 (n = 16). In addition, digestive content samples (*i.e.*, gastrointestinal content) were collected from 10 striped dolphins (5 females and 5 males) stranded during the most recent period. The cause of death for most dolphins was related to the morbillivirus epizootics that occurred in the Mediterranean Sea during 1990 and 2007–08. That of the remaining dolphins was hardly determined. Age information was not available, however, only adult individuals, whose body length was higher than 1.8 m, were included in the study (Table S1). The samples were preserved frozen (−20 °C) in the biological tissue bank of the University of Barcelona until analysis. Subsamples of approximately 2 g of frozen liver and digestive content were lyophilized for 48 h.

### 2.2. Sample treatment

Standards and reagents are listed in Annex 1 (SI).

The analysis of PFAS in the digestive content and liver of striped dolphins was performed according to a previously published study by Roscales et al. (2022). Briefly, 0.3 g dry weight sample was transferred into 15 mL polypropylene (PP) tubes and spiked with 50 μL of a surrogate solution containing $^{13}$C-labeled perfluorohexanoic acid (PFHxA), PFOA, perfluorononanoic acid (PFNA), perfluorooctanesulfonamide

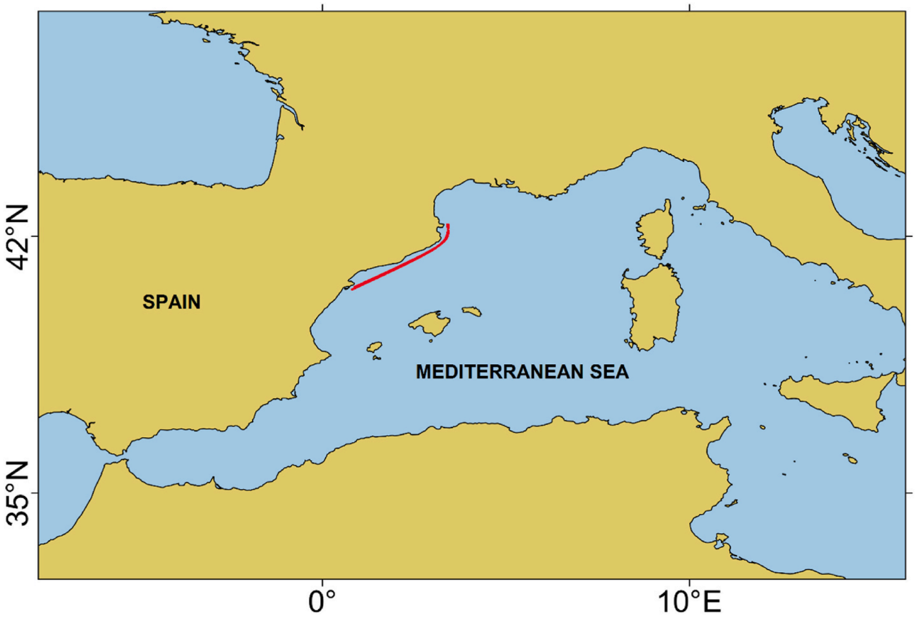

**Fig. 1.** Map of the Western Mediterranean Sea highlighting (in red) the area of the Spanish Catalan coast (NW Mediterranean Sea), where the striped dolphins, sampled in this study, stranded. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(FOSA), PFOS, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), and $^{18}$O-labeled perfluorohexanesulfonic acid (PFHxS) (Wellington Laboratories, Ontario, Canada) in methanol at a concentration of 50 pg/μL. After 1 h of equilibration, a liquid-solid extraction was carried out using 9 mL of 0.01 M sodium hydroxide in methanol solution under constant agitation for 16 h. The supernatant (3 mL) was collected after centrifugation (10 min at 4000 rpm) and transferred into new PP tubes along with 27 mL of chromatographic-grade water. The sample was then purified using Oasis WAX cartridges (150 mg, Waters) previously conditioned with 4 mL methanol, 4 mL ammonia (0.1% in methanol), and 4 mL of chromatographic-grade water. The sample elution was facilitated using a Baker® station under low vacuum conditions at a constant slow flow (>15 ml/min). Each cartridge was pH conditioned with 4 mL of 25 mM ammonium acetate buffer at pH 4 and dried under vacuum aspiration for 30 min. Elution was performed using 4 mL methanol and 4 mL ammonia (0.1% in methanol), concentrated under a gentle nitrogen flux to approximately 0.5 mL, and then transferred to self-filtration PP vials (Whatman). After drying, each vial was reconstituted with methanol, and 30 μL were transferred to an injection vial along with 30 μL of an injection standard solution containing $^{13}C_8$-labeled PFOA and PFOS (Wellington Laboratories, Ontario, Canada), at a concentration of 10 pg/μL in methanol, and 140 μL of chromatographic-grade water.

### 2.3. Instrumental analysis

The determination of PFAS was performed following the methodology described by Roscales et al. (2022). The Ultra Performance Liquid Chromatography-triple-quadrupole mass spectrometer (UPLC-MS/MS) system (Waters Acquity-Waters XEVO TQS, Waters, Milford, MA) was used in multiple-reaction monitoring (MRM) mode and equipped with an electrospray ionization (ESI) source in negative mode. The source temperature was set to 150 °C, and the capillary and cone voltages were adjusted between 3.50 and 3.05 Kv, and between 30 and 77 V, respectively. The desolvation temperature was 450 °C, and the gas flow was set to 900 L/h. The collision gas flow was set between 0.16 and 0.15 mL/min and the nebulizer gas flow pressure was between 7.00 and 5.84 bar. The samples were maintained at 4 °C and 10 μL of each sample was injected into the UPLC-MS/MS system at a rate of 30 μL/min.

The UPLC column used was an Acquity UPLC BEH C18 column (1.7 μm, 2.1 mm × 100 mm; Waters), which was maintained at a constant temperature of 50 °C and a flow rate of 0.3 mL/min. The mobile phase consisted of a constant 1% ammonium acetate buffer in water (200 mM, pH 4) and the organic phase for analysis was a mixture of methanol and acetonitrile (80:20). The gradient water:organic phase for each 18-min injection was 60:40 at the initial conditions, 45:55 at 3.5 min, 30:70 at 10 min, 15:85 at 15 min, and returned to the initial conditions from 17.5 min. A total of 19 PFAS were identified and quantified, as listed in Tables S1 and S2. Linear PFOS is indicated as n-PFOS and the term Br-PFOS has been used to express the sum of all branched isomers quantified here.

### 2.4. Quality assurance

To avoid contamination with fluorinated substances, appropriate precautions were taken during both field and laboratory procedures. All polypropylene materials were cleaned with methanol, and glass materials were muffled at 500 °C for 24 h prior to use. The identification of target compounds was based on their retention times and the ratio between the monitored ions, which should match the values in the calibration curves within ±30%. Each sample was injected twice, with the mean value reported. A procedural blank was conducted for every batch of nine samples. Limits of detection (LODs) and limits of quantification (LOQs) were calculated either as the mean concentration of each compound in the blanks or as the average background noise plus 3 and 10 times the standard deviation, respectively. No blank corrections were performed. LODs, LOQs, and recoveries are summarized in Tables S3 and S4 (SI). All PFAS were found to have satisfactory recoveries (75–110%), except for PFBA, which was excluded from the results due to unreliable recoveries of $^{13}C_4$-PFBA and impurity co-elutions in several samples.

### 2.5. Statistical analysis

The normality and heteroscedasticity of each target analyte were evaluated using the Shapiro-Wilk and Levene tests, respectively. As the data did not follow a normal distribution, non-parametric tests were used to analyse each PFAS individually. The presence of outliers was tested graphically through boxplots. One sample of digestive content (N_408_18_E) was excluded from the statistical analysis because it contained 5 different PFAS compounds that exceeded the 3* interquartile range limit.

The samples collected in the years 2014–2021, which included 16 liver samples, and 9 digestive content samples (5 from male and 4 from female dolphins), were used to carry out a series of comparisons using the Kruskall Wallis test. First, PFAS concentrations of the digestive contents were compared between sexes to assess the possibility to pool the values of males and females in a homogeneous group. Then, the prevalence of n-PFOS, with respect to the total PFOS (*i.e.*, n-PFOS plus Br-PFOS), was compared between the digestive contents and the liver of the dolphins. Finally, the concentrations of each compound were compared between the liver and the digestive content.

Subsequently, the biomagnification factors (BMFs, *i.e.*, concentration in predator/concentration in prey (Houde et al., 2011)) were calculated as the PFAS concentration in the liver of the dolphins divided by the average of PFAS concentrations in their digestive contents (*i.e.,* no matching pairs). This approach is limited by the classical challenge of using stomach contents, as it only reflects the immediate short-term diet (*i.e.*, the last meal) and not the main prey in the long term (Alfaro Garcia et al., 2022).

Finally, a Kruskal-Wallis test and a Dunn's Test using the Benjamini-Hochberg method were conducted to determine the influence of the year of sampling on PFAS concentrations in the liver of striped dolphins collected between the years 1990–2021 ($n = 48$), which were divided into three groups: 1990 ($n = 19$), 2004–2009 ($n = 13$) and 2014–2021 ($n = 16$).

Values below the LOD were considered half of the LOD. Compounds that were detected with a frequency less than 50%, were excluded from the statistical analysis (*i.e.*, perfluorobutane sulfonic acid (PFBS), Perfluorodecane sulfonic acid (PFDS), perfluorododecane sulfonic acid (PFDoDS), perfluoropentanoic Acid (PFPA), PFHxA, perfluoroheptanoic acid (PFHpA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFOcDA)). The significance level was set at $p < 0.05$, and all analyses were performed using R (R Core Team, 2023).

## 3. Results

No significant differences were detected in PFAS concentrations between the digestive contents of males and females, so samples from both sexes were pooled in a homogeneous group.

PFAS concentration of each analysed compound per individual digestive content and liver sample is shown in Table S1. Median, minimum, and maximum concentrations of each target compound in digestive content and liver samples of striped dolphins are summarized in Table 1. PFAS were detected in all samples, at concentrations ranging between 43 and 1609 ng/g in the digestive content samples and between 254 and 7010 ng/g in the liver samples (Table 1). Out of 19 targeted PFAS, 13 were detected in the digestive content samples and 17 in the liver samples. n-PFOS and FOSA were the most abundant compounds detected in 100% of both types of samples (Table 1). The composition of PFAS consisted mainly of the same five dominant PFAS: n-PFOS > FOSA > PFUnDA ≈ perfluorotridecanoic acid (PFTrDA) > PFNA (Fig. 2). The

**Table 1**

PFAS concentrations expressed in ng/g wet weight (median, minimum, maximum), frequency of occurrence (FO; %), and n-PFOS prevalence (%; n-PFOS/PFOS*100) from samples of striped dolphins from the NW Mediterranean Sea. Data from digestive content refer to the 2014–2018 period, and liver data to the 1990–2021 period. Biomagnification factor (BMF) ranges for each PFAS compound are also shown (only for those PFAS quantified in more than 50% of the samples).

| Compound | Striped dolphin digestive content | | | | Striped dolphin liver | | | | BMF |
|---|---|---|---|---|---|---|---|---|---|
| | Median | Min | Max | FO | Median | Min | Max | FO | |
| **PFBS** | nd | nd | nd | 0 | nd | nd | 19.49 | 8.33 | – |
| **PFHxS** | nd | nd | 53.78 | 11.11 | 3.99 | nd | 26.56 | 70.83 | 0.03–2.45 |
| **n-PFOS** | 34.96 | 5.58 | 444.03 | 100 | 920.81 | 95.85 | 3964.72 | 100 | 4.96–15.47 |
| **Br-PFOS** | 21.36 | 2.29 | 645.67 | 100 | 27.54 | 2.46 | 115.31 | 100 | 0.14–1.11 |
| **n-PFOS (%)** | 71.96 | 34.74 | 92.31 | – | 97.19 | 91.38 | 98.37 | – | – |
| **PFDS** | nd | nd | 6.89 | 11.11 | nd | nd | 19.97 | 20.83 | – |
| **PFDoDS** | nd | nd | nd | 0 | nd | nd | 16.31 | 2.08 | – |
| **FOSA** | 59.11 | 16.00 | 431.50 | 100 | 404.87 | 44.75 | 2055.08 | 100.00 | 0.41–18.76 |
| **PFPA** | nd | nd | nd | 0 | nd | nd | 422.46 | 14.58 | – |
| **PFHxA** | nd | nd | nd | 0 | nd | nd | 225.85 | 12.50 | – |
| **PFHpA** | nd | nd | 17.60 | 11.11 | 4.20 | nd | 51.68 | 25 | – |
| **PFOA** | 1.30 | nd | 6.00 | 55.56 | 9.29 | 1.16 | 42.48 | 100 | 2.45–14.57 |
| **PFNA** | 5.72 | 1.45 | 78.05 | 100 | 59.90 | 1.50 | 319.77 | 100 | 3.70–19.45 |
| **PFDA** | 7.27 | 0.97 | 90.31 | 100 | 69.07 | 3.19 | 228.68 | 100 | 3.51–13.47 |
| **PFUnDA** | 14.54 | 3.06 | 160.10 | 100 | 191.93 | 5.73 | 959.85 | 100 | 4.72–12.46 |
| **PFDoDA** | 3.28 | 1.18 | 42.28 | 100 | 44.35 | 2.09 | 215.25 | 100 | 2.33–10.35 |
| **PFTrDA** | 13.37 | 3.61 | 168.61 | 100 | 149.03 | 6.74 | 1105.19 | 100 | 3.41–17.29 |
| **PFTeDA** | 2.70 | nd | 31.77 | 55.56 | 15.03 | nd | 197.19 | 81.25 | 0.17–20.56 |
| **PFHxDA** | nd | nd | nd | 0 | nd | nd | nd | 0 | – |
| **PFOcDA** | nd | nd | nd | 0 | nd | nd | nd | 0 | – |
| **∑PFAS** | 158.76 | 42.96 | 1609.26 | 100 | 2326.03 | 254.23 | 7010.44 | 100 | – |

nd: non-detected compound, below the limit of detection.

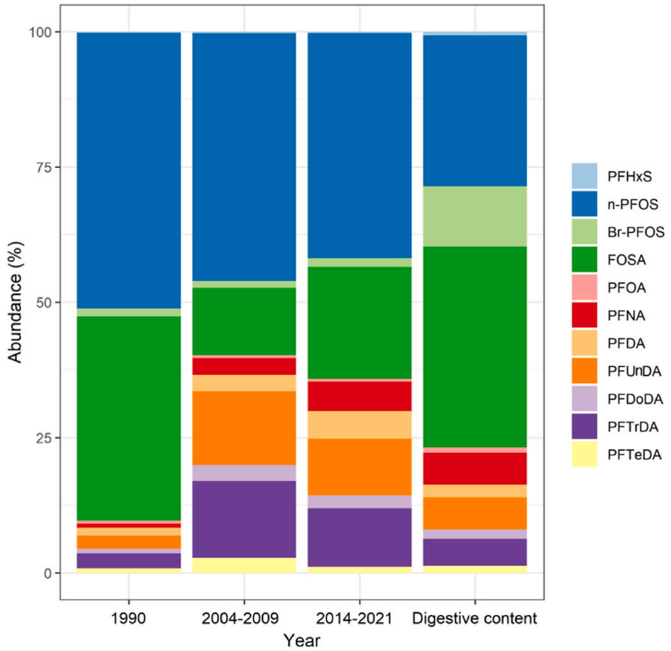

**Fig. 2.** Relative contribution of detected PFAS split by year of collection in the liver (n = 48) and digestive content (n = 9) of striped dolphins stranded along the Spanish Catalan coast (NW Mediterranean Sea). Only the PFAS that were quantified in over 50% of the samples are shown.

relative contribution of n-PFOS to the total amount of PFOS was significantly lower in the digestive content samples (median = 72%) than in the liver samples (median = 97%) (Table 1). All PFAS that were detected in over 50% of the samples were long-chain PFAS.

When considering only the samples collected between 2014 and 2021, the concentrations of n-PFOS, FOSA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFTrDA were significantly lower in digestive content samples than in liver samples (Fig. 3, Table 1), while the concentrations of PFHxS, Br-PFOS and perfluorotetradecanoic acid (PFTeDA) did not show significant differences between the two sample types (Fig. 3, Table 1).

The mean BMFs for n-PFOS, FOSA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFTrDA were 9.91, 5.11, 7.36, 9.03, 8.08, 8.43, 6.66 and 9.52, respectively. The mean BMFs for PFHxS, Br-PFOS and PFTeDA were 0.86, 0.42 and 5.25, respectively. BMF ranges for each PFAS compound are shown in Table 1.

The Kruskal-Wallis and Dunn's tests performed on the liver samples of striped dolphins sorted by the sampling period (1990, 2004–2009 and 2014–2021) indicated a significant increase in the concentration of n-PFOS, Br-PFOS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFTrDA from 1990 to 2004–2009, and a subsequent stabilization during the following years (2014–2021, Fig. 4). However, no significant time trend was observed in the concentrations of PFHxS and FOSA, while a significant increase (from 1990 to 2004–2009) followed by a significant decrease (from 2009 to 2014–2021) was detected in the concentrations of PFTeDA (Fig. 4).

## 4. Discussion

In this study, we investigated PFAS hepatic concentrations in striped dolphins from the NW Mediterranean Sea over a 30-year (from 1990 to 2021) period, the potential biomagnification of PFAS concentrations from prey to striped dolphins, and the temporal trends of these pollutants in the striped dolphin liver.

### *4.1. PFAS concentrations and profiles*

Consistently to the current results, PFOS is the predominant PFAS compound in marine biota (Andvik et al., 2023; Cara et al., 2022; Colomer-Vidal et al., 2022; Kelly et al., 2009). Data about PFAS in marine mammals from the Mediterranean Sea are scarce. To our knowledge, only three studies have been published to date. Kannan et al. (2002) were the first to report PFAS concentrations (n-PFOS, FOSA, PFOA, and PFHxS) in cetaceans from the Mediterranean Sea (bottlenose dolphins (*Tursiops truncatus*), common dolphins (*Delphinus delphis*), long-finned pilot whales (*Globicephala melas*), and striped dolphins). Consistently with our results, they found n-PFOS to be the most abundant PFAS in the liver of striped dolphins, although they didn't detect either FOSA or PFOA. They reported a median concentration of n-PFOS

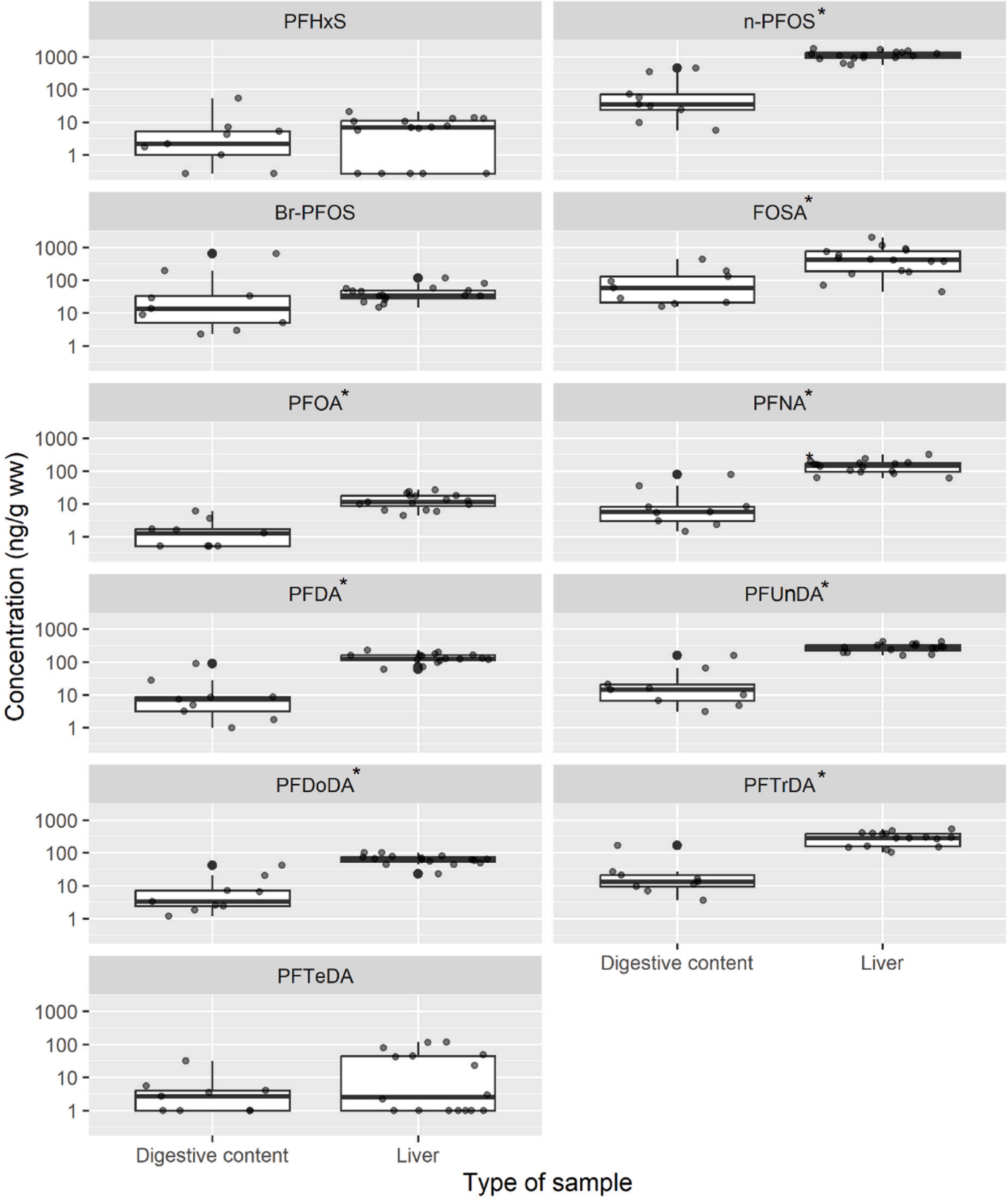

**Fig. 3.** Box-plot illustrating PFAS concentrations in the digestive content (n = 9, year of collection: 2014–2018) and in the liver (n = 16; year of collection: 2014–2021) samples of striped dolphins from the NW Mediterranean Sea. Boxes represent the first and third quartiles, horizontal lines the median, and vertical bars indicate the most extreme data point that is lower than 1.5 times the interquartile range from the box. Asterisks (*) indicate significant differences between the two types of samples (Kruskal-Wallis test). Only the PFAS that were quantified in over 50% of the samples are shown.

lower than the concentration we detected (22.90 and 920.81 ng/g wet weight (ww), respectively), probably indicating regional differences in PFAS inputs between the Spanish Catalan coast and the Italian coast. This could be a feasible explanation, since striped dolphin home ranges typically extend over distances ranging from 200 to 500 km (Costantino et al., 2019), and thus the two populations likely are segregated, showing different pollutant concentrations. On the other hand, Kannan et al. (2002) reported a n-PFOS concentration in the liver of common dolphins similar to the median value we found in the liver of striped dolphins (940 and 920.81 ng/g ww, respectively). López-Berenguer et al. (2020) analysed twelve different PFAS in the muscle and liver of striped dolphins, bottlenose dolphins, common dolphins, Risso's dolphins (*Grampus griseus*), and sperm whales (*Physeter macrocephalus*) from the SW Mediterranean Sea. Similarly to our results, they found that the PFAS profile in both the liver and muscle of the five studied cetacean species was mainly composed of five dominant PFAS: n-PFOS > FOSA > PFNA ≈ PFUnDA > PFDA, indicating that they probably came from similar sources. The median concentrations of these PFAS in the liver of

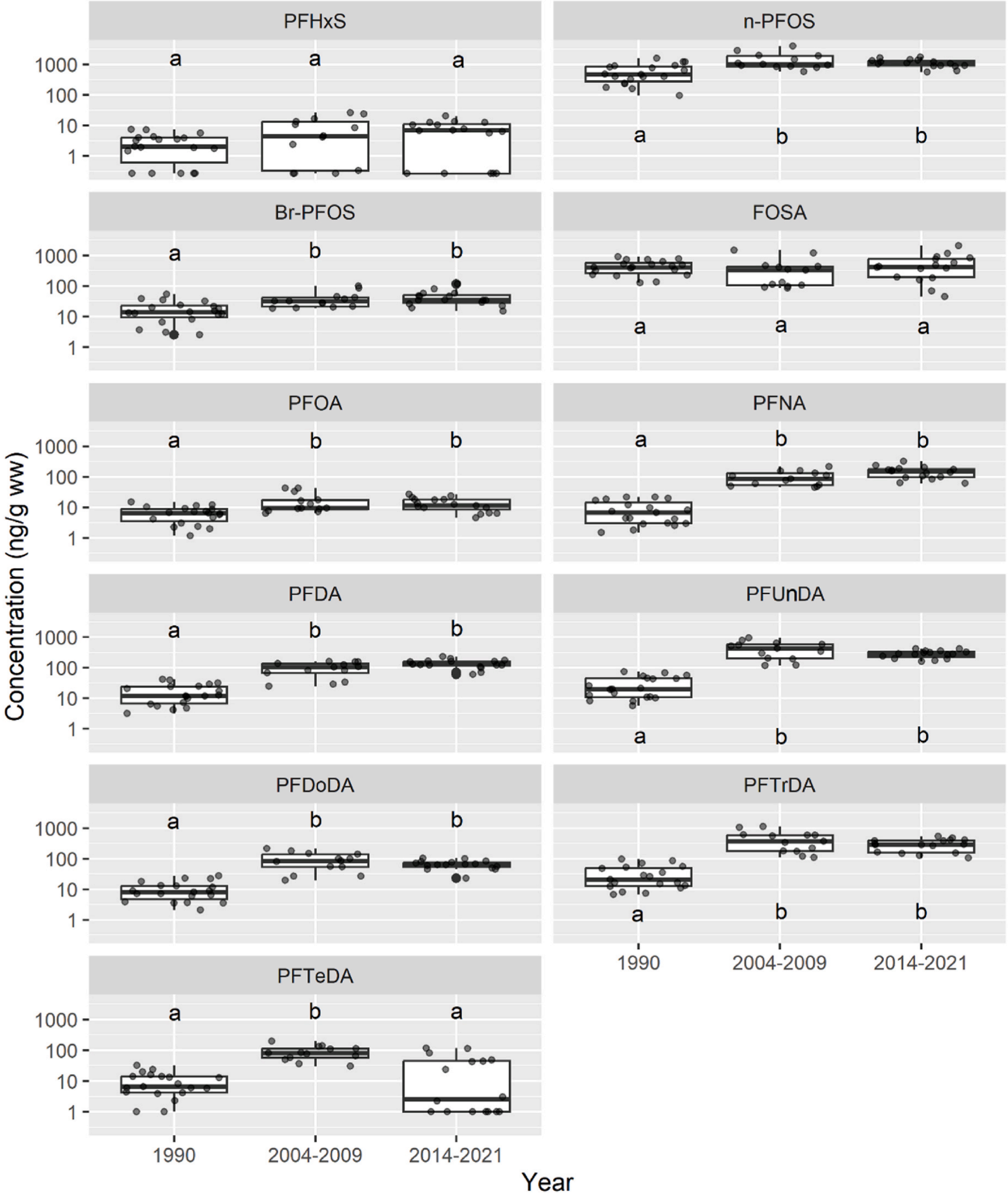

**Fig. 4.** Box-plot illustrating PFAS concentrations in liver (n = 48) samples of striped dolphins from the NW Mediterranean Sea split by the period of collection (1990, 2004–2009, and 2014–2021). Boxes represent the first and third quartiles, horizontal lines the median, and vertical bars indicate the most extreme data point that is lower than 1.5 times the interquartile range from the box. Boxplots indicated by different lowercase letters are statistically different in their median values according to Kruskal-Wallis and Dunn's tests. Only the PFAS that were quantified in over 50% of the samples are shown.

striped dolphin reported by the authors was lower than the values we found, probably indicating a higher pollution impact by PFAS in the NW Mediterranean Sea than in the SW Mediterranean Sea. Sciancalepore et al. (2021) analysed 17 target PFAS in the liver of bottlenose dolphins stranded in the Adriatic Sea. The PFAS profile was mainly composed of the same five dominant PFAS: n-PFOS > PFUnDA > PFDA ≈ PFDoA ≈ PFTrDA, which is similar to that reported in the current study, except for FOSA, which was not relevant for them. The authors found a median n-PFOS concentration six times lower than the concentrations we found (152.21 and 920.81 ng/g ww, respectively), likely indicating differences between species and areas, as reported before (Kannan et al., 2002; López-Berenguer et al., 2020). On the other hand, while PFHxS, PFHpS, n-PFOS, PFDS, FOSA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and PFTeDA have been previously detected in the liver of striped dolphins from the Mediterranean Sea (Kannan et al., 2002; López-Berenguer et al., 2020), Br-PFOS, PFPA, PFHxA, and PFHpA have never been reported before the current study. The high concentrations of FOSA reported in the current study in comparison to the other studies of PFAS in marine mammals from the Mediterranean Sea (Kannan et al., 2002; López-Berenguer et al., 2020; Sciancalepore et al., 2021) deserve further research. However, the concentrations of FOSA in the blood of bottlenose dolphins and swordfish inhabiting the Mediterranean Sea surpassed the corresponding levels of n-PFOS (Kannan et al., 2002). This can be attributed to the absence of a pivotal metabolic enzyme in cetaceans, which is essential for the conversion of FOSA into PFOS (Letcher et al., 2014).

A study on the baseline levels of PFAS in the open Western Mediterranean Sea waters shows that PFHxA, PFHpA, PFOA, PFHxS and PFOS are the most abundant compounds (Brumovský et al., 2016). This indicate that short-chain PFAS dominate the baseline of trophic chains (Diao et al., 2022), but as reported in the current study, just long-chain PFAS biomagnify to top predators.

Our study clearly evidences the prevalence of the linear isomers, consistent with previous research, suggesting a preferential accumulation of linear PFOS isomers over their branched counterparts (Schulz et al., 2020; Sharpe et al., 2010). Specifically, studies in fish (Roscales et al., 2022) and marine mammals (Rotander et al., 2012; Stockin et al., 2021) reported a high prevalence of n-PFOS, in line with our own results. A plausible explanation for the higher prevalence of n-PFOS in the liver of dolphins from the current study may be attributed to its higher bioaccumulation potential and longer half-life compared to Br-PFOS (Sharpe et al., 2010).

### 4.2. Biomagnification of PFAS

The evidence supporting the occurrence of PFAS biomagnification is substantial and well-documented across multiple studies (Houde et al., 2006; Kannan et al., 2005; Kelly et al., 2009; Martin et al., 2004; Tomy et al., 2004), although biomagnification patterns of PFAS are complex and likely affected by several factors, such as ecological characteristics, biotransformation, and the physicochemical properties of both PFAS and the environmental compartments (*i.e.*, sediment and water (Cara et al., 2022). PFAS disposal pathways are inefficient (Ma et al., 2020; Zhang et al., 2015). PFAS elimination via the lung to air is limited, resulting in higher biomagnification of PFAS in air-breathing mammals such as dolphins (Bossi et al., 2005; Houde et al., 2006; Khan et al., 2023) than, for instance, in fish (Cara et al., 2022; Kelly et al., 2009). It should be noted that our results must be interpreted with caution, as we are comparing digestive content, on a specific day, with hepatic PFAS concentrations, and the diet of striped dolphins is highly diverse, commonly exploiting a wide variety of oceanic, pelagic and bathypelagic prey species (Aguilar, 2000; Saavedra et al., 2022), which surely is not reflected in the stomach content of a specific day. Moreover, we are comparing the digestive content of different dolphins than the ones from which we analysed the liver. This adds a further bias, although to a lesser extent than the issue of the stomach content reflecting only the diet of a specific moment.

Martin et al. (2004) estimated BMFs for FOSA, PFOS, and PFCAs (8–15 carbons) in a food web from Lake Ontario with lake trout (*Salvelinus namaycush*) as the top predator, ranging from 0.51 for FOSA to 5.88 for n-PFOS. Tomy et al. (2004) evaluated the TBFs for n-PFOS, PFOA, FOSA and N-etPFOSA in an Arctic marine food web with belugas (*Delphinapterus leucas*) and narwhals (*Monodon monoceros*) as top predators, and found TBFs ranging from 0.9 to 9 for PFOS and often BMFs higher than 1 for the rest of the compounds. Kelly et al. (2009) also assessed biomagnification of n-PFOS, FOSA, and PFCAs (7–14 carbons) in an Arctic marine food web with belugas as top predators, and found BMFs of perfluorinated acids ranging between 2 and 11. Houde et al. (2006) studied a marine food web with bottlenose dolphins as top predators and reported BMFs of PFOS and PFCAs (8–14 carbons) ranging from 1 to 156 and from 1 to 30 in Sarasota Bay and Charleston Harbour, respectively. Consistently, in the current study, 8 of the 11 most abundant detected PFAS biomagnified, with mean BMFs ranging from 5.11 for FOSA to 9.91 for n-PFOS. Indeed, all PFAS showing biomagnification were long-chain PFAS ($\geq$6 carbons (Brendel et al., 2018), which is consistent with studies showing increasing BMF in PFAS with increasing carbon chain length in the $C_6$–$C_{13}$ range (Kannan et al., 2005; Tomy et al., 2004). On the other hand, in the ecosystem of the Yadkin-Pee Dee River (US), PFBS, a short-chain PFAS, showed some BMFs exceeding 1 (Penland et al., 2020). The authors explained these unexpected results by hypothesizing that an unmeasured PFBS precursor may be accumulating in biota and metabolizing to PFBS. Moreover, in a terrestrial food web of the Tibetan Plateau, PFBA (BMF = 5.11) and PFBS (BMF = 5.96), along the plant-pika-eagle trophic pathway, exhibit BMFs significantly exceeding 1 (Huang et al., 2022). The pronounced biomagnification of the short-chain PFAS in air-breathing organisms may be attributed to their absence of biotransformation and resistance to volatilization (Huang et al., 2022).

Despite a transition towards the utilization of shorter-chain PFAS such as PFBA and PFBS, long-chain PFAS, including PFOS, PFOA, PFTrDA, and PFUnDA, continue to account for approximately 75% of PFAS, with PFOS contributing more than 35%, in higher trophic level organisms such as cephalopods, crustaceans, and fish in Qinzhou Bay (Du et al., 2021).

As PFAS biomagnification clearly occurs in their tissues, the ecological risks of PFAS for striped dolphins should be considered. Environmental Quality Standards (EQSs) refer to established limits of concentration for a specific pollutant or set of pollutants in water, sediment, or biota that must not be surpassed to safeguard both human health and the environment. An EQS was established in 2013 by the European Union under the Water Framework Directive (European Union (EU), 2013). A value of 9.1 ng PFOS/g ww in humans was set to protect aquatic food webs and top predators. Values detected in all the liver samples and in 8 out of 9 digestive content samples analysed here exceeded the EQS, indicating potential health risks for striped dolphins inhabiting the NW Mediterranean Sea. Indeed, *in vitro* studies demonstrated cell cycle alterations and gene expression changes due to the interaction of PFAS with bottlenose dolphin skin cells, confirming the toxicity of PFAS for dolphins (Mollenhauer et al., 2009; Otero-Sabio et al., 2022). Furthermore, a Quality Standard benchmark of 33 ng PFOS/g ww in prey was set in 2014 for the protection of wildlife against secondary poisoning (*i.e.*, poisoning through a contaminated prey) (European Union (EU), 2014). This concentration was exceeded in half of the samples of digestive content, suggesting that polluted prey may pose potential health risks for striped dolphins. The Mediterranean striped dolphin primarily feeds on mesopelagic and neritic fish species, as well as oceanic squids (Aguilar, 2000; Saavedra et al., 2022). Their preferred prey includes cephalopods from the families Ommastrephidae, Histiotheuthidae, Enoploteuthidae and Onychoteuthidae, as well as bony fishes from the families Mictophidae, Gadidae, Sparidae and Gonostomiatidae (Aguilar, 2000; Saavedra et al., 2022). Most of these cephalopod and bony fish families comprise commercial species consumed globally, thereby also potentially posing health risks to humans. The species composition of the digestive content was not analysed in the current study.

### 4.3. Temporal trends

Although our temporal analyses were based only on 48 samples and thus the observed trends must be interpreted with caution, the present long-term study, covering over three decades, showed an increase in PFAS concentration between 1990 and 2004–2009, followed by a stabilization in the most recent years (2014–2021). Long-chain PFAS, including PFCAs (with 7 or more carbon atoms) and PFSAs (with 6 or more carbon atoms), are of particular concern due to their high persistence, bioaccumulation, and ubiquity in the environment and biota (Conder et al., 2008; Giesy and Kannan, 2001; Parsons et al., 2008; Rayne and Forest, 2009). Since 2009, some of these substances have been listed as restricted under the Stockholm Convention and the European chemicals regulation (REACH) due to their adverse effects on humans and the environment (ECHA, E.C.A., 2013). The results of the current study also align with industry initiatives to reduce the use of long-chain PFAS, such as replacing them with their shorter-chain homologues or other types of (non)fluorinated chemicals (3 M, 2000; Ritter, 2010). For example, in the year 2000, the 3M Company initiated a global phase-out of its long-chain PFAS products and replaced them with products based on $C_4$ chemistry, such as PFBS (3 M, 2000). In 2006, eight major producers of PFAS joined the US Environmental Protection Agency (EPA) PFOA Stewardship Program with the aim of eliminating long-chain PFAS and their potential precursors by 2015 (US EPA, 2006). It is important to note that shorter-chain PFAS, such as PFBS ($C_4$) or PFHxA ($C_6$), may not be detectable due to their lower capacity for bioaccumulation in biota (Renner, 2006). Accordingly, despite short-chain PFAS are replacing long-chain PFAS in the industry, the present results did not show any trend for short-chain PFAS in the recent samples of striped dolphins. Therefore, the striped dolphin is not a good bioindicator to assess temporal trends of short-chain PFAS in the marine environment.

While in isolated regions, such as the Arctic, PFAS mainly derive from the oceanic currents and atmospheric transport of volatile precursors (Yeung et al., 2017), river discharges are the primary sources of PFAS in the Mediterranean basin (Brumovský et al., 2016). The Mediterranean Sea is also susceptible to significant environmental contamination due to its semi-enclosed geomorphology and high level of coastal human activity (Ahrens et al., 2010; Kurwadkar et al., 2022; Zhang et al., 2017). The Mediterranean Sea has been shown to be more polluted in coastal areas than in the pelagic waters and in surface waters compared to deep waters, due to inland PFAS sources (Brumovský et al., 2016). Significant amounts of PFAS have been detected in large Mediterranean rivers, including the Ebre, Llobregat, and Po rivers. (Campo et al., 2015; Lorenzo et al., 2016; McLachlan et al., 2007; Pignotti et al., 2017). The absence of changes could be attributed to the fact that the decrease in PFAS contributions to the sea has not yet been reflected in the concentrations of dolphins due to the high persistence of these compounds.

Although the production of long-chain PFAS (including PFOS and PFOA) was reduced almost two decades ago in the US and Western Europe (Paul et al., 2009), they are still the most detected PFAS compounds in biota (López-Berenguer et al., 2020; Sun et al., 2019). This is mainly due to their high persistence and prevalence in the environment, the fact that they can be formed from precursors (Wang et al., 2017), their high potential for bioaccumulation (Kannan et al., 2005), and their continued production in Asian countries, particularly in China (Wang et al., 2014). In line with our results, Rotander et al. (2012) analysed several species of marine mammals from the North Atlantic Ocean and detected an increase in the levels of long-chain PFCAs ($C_9$–$C_{12}$) from 1984 to 2009, although the levels of PFOS remained steady during the studied period. Galatius et al. (2011) also found increasing trends of long-chain PFCAs (PFUnDA and PFDA) from 1980 to 2005 in the liver of harbour porpoises (*Phocoena phocoena*) from the North Sea. Lam et al. (2016) reported significant increasing trends of PFCAs and PFBS in marine mammals from the South China Sea from 2002 to 2014 and attributed this pattern to the restriction of the production of PFOS.

On the other hand, some temporal studies have reported a decrease in PFOS concentrations in biota over time (*e.g.*, Colomer-Vidal et al., 2022; Houde et al., 2011). For instance, in the Netherlands, PFOS levels in European eel (*Anguilla anguilla*) decreased by a factor of 2–4 from the 1990s to 2008 (Kwadijk et al., 2010). Similarly, Munschy et al. (2019) found a significant decrease in PFOS levels in shellfish from French coasts after the mid-1990s. This opposite trend compared with the results presented here may be a consequence of local management practices or specific regional characteristics.

## 5. Conclusions

In this study we detected 17 PFAS, 11 of which in over half of the hepatic samples of striped dolphins analysed. These results underscore the enduring existence of some regulated PFAS such as PFOS, PFOA and PFHxS. Most of the detected PFAS biomagnified, posing potential health risks to striped dolphins. All liver samples and eight digestive content samples exceeded the Quality Standard for PFOS, indicating that prey items consumed by striped dolphins, consisting mainly of commercially exploited and globally consumed fish and cephalopod species, may also potentially expose humans to health risks.

This study represents one of the most extensive long-term analyses of PFAS in a marine apex predator inhabiting a pollution hotspot and reveals a significant increase in the concentration levels of most long-chain PFAS from 1990 to 2004–2009 followed by a stabilization during the more recent period (2014–2021). These trends are consistent with the global release of PFAS, their persistent and ubiquitous nature in the environment, and the efforts towards their mitigation that have been implemented over the last two decades.

Although global emissions of long-chain PFAS have reduced in recent years across most countries, these pollutants remain in the environment due to their high persistence. The key findings of this study have important implications for future ecological risk assessments and can inform the development of management strategies aimed at mitigating PFAS pollution in marine ecosystems. Both national and international regulations should be intensified and reinforced, and industry initiatives should aim to reduce the use of long-chain PFAS and replace them with safer alternatives. This may help to mitigate the adverse effects of PFAS on the environment and biota.

On the other hand, it is critical to continue monitoring the levels of PFAS in the environment and biota to evaluate the effectiveness of the phase-out and substitution of long-chain PFAS. Additionally, more research is needed to investigate the potential adverse effects of shorter-chain PFAS and other alternative chemicals that are being used in place of long-chain PFAS.

## CRediT authorship contribution statement

**Odei Garcia-Garin:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Asunción Borrell:** Conceptualization, Writing – review & editing, Supervision, Resources. **Pere Colomer-Vidal:** Methodology, Supervision, Writing – review & editing. **Morgana Vighi:** Writing – review & editing, Supervision. **Núria Trilla-Prieto:** Methodology, Writing – review & editing. **Alex Aguilar:** Resources. **Manel Gazo:** Writing – review & editing, Resources. **Begoña Jiménez:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## 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.

## Data availability

Data will be made available on request.

## Acknowledgements

The authors thank the MCIN/AEI/10.13039/501100011033 and FSE “El FSE invierte en tu futuro” for supporting O. Garcia-Garin with a Ph. D. FPU scholarship (FPU17/00073) and with a Margarita Salas grant funded by the Spanish Ministry of Universities and the European Union Next Generation EU/PRTR, as well as the recovery, transformation and resilience plan, and the complementary call for applications for grants for the requalification of the Spanish university system for the period 2021–2023.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.122738.

## References

3 M, 2000. Letter to US EPA, Re: Phase-Out Plan for POSF-Based Products, pp. 226–600.

Aguilar, A., 2000. Population biology, conservation threats and status of Mediterranean striped dolphins (*Stenella coeruleoalba*). J. Cetacean Res. Manag. 2, 17–26.

Ahrens, L., Gerwinski, W., Theobald, N., Ebinghaus, R., 2010. Sources of polyfluoroalkyl compounds in the North Sea, Baltic Sea and Norwegian Sea: evidence from their spatial distribution in surface water. Mar. Pollut. Bull. 60, 255–260. https://doi.org/10.1016/j.marpolbul.2009.09.013.

Alfaro Garcia, L.A., Descamps, S., Herzke, D., Chastel, O., Carravieri, A., Cherel, Y., Labadie, P., Budzinski, H., Munoz, G., Bustamante, P., Polder, A., Gabrielsen, G.W., Bustnes, J.O., Borgå, K., 2022. Bioaccumulation of per and polyfluoroalkyl substances in Antarctic breeding South polar Skuas (*Catharacta maccormicki*) and their prey. Front. Mar. Sci. 9 https://doi.org/10.3389/fmars.2022.819525.

Andvik, C., Haug, T., Lyche, J.L., Borgå, K., 2023. Emerging and legacy contaminants in common minke whale from the Barents sea. Environ. Pollut. 319 https://doi.org/10.1016/j.envpol.2023.121001.

Berthiaume, J., Wallace, K.B., 2002. Perfluorooctanoate, perflourooctanesulfonate, and N-ethyl perfluorooctanesulfonamido ethanol; peroxisome proliferation and mitochondrial biogenesis. Toxicol. Lett. 129, 23–32. https://doi.org/10.1016/S0378-4274(01)00466-0.

Bossi, R., Riget, F.F., Dietz, R., Sonne, C., Fauser, P., Dam, M., Vorkamp, K., 2005. Preliminary screening of perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish, birds and marine mammals from Greenland and the Faroe Islands. Environ. Pollut. 136, 323–329. https://doi.org/10.1016/j.envpol.2004.12.020.

Brendel, S., Fetter, É., Staude, C., Vierke, L., Biegel-Engler, A., 2018. Short-chain perfluoroalkyl acids: environmental concerns and a regulatory strategy under REACH. Environ. Sci. Eur. 30, 9. https://doi.org/10.1186/s12302-018-0134-4.

Brumovský, M., Karásková, P., Borghini, M., Nizzetto, L., 2016. Per- and polyfluoroalkyl substances in the western Mediterranean Sea waters. Chemosphere 159, 308–316. https://doi.org/10.1016/j.chemosphere.2016.06.015.

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integrated Environ. Assess. Manag. 7, 513–541. https://doi.org/10.1002/ieam.258.

Campo, J., Pérez, F., Masiá, A., Picó, Y., Farré, Marinel la, Barceló, D., 2015. Perfluoroalkyl substance contamination of the Llobregat River ecosystem (Mediterranean area, NE Spain). Sci. Total Environ. 503 (504), 48–57. https://doi.org/10.1016/j.scitotenv.2014.05.094.

Cara, B., Lies, T., Thimo, G., Robin, L., Lieven, B., 2022. Bioaccumulation and trophic transfer of perfluorinated alkyl substances (PFAS) in marine biota from the Belgian North Sea: distribution and human health risk implications. Environ. Pollut. 311, 119907 https://doi.org/10.1016/j.envpol.2022.119907.

Colomer-Vidal, P., Bertolero, A., Alcaraz, C., Garreta-Lara, E., Santos, F.J., Lacorte, S., 2022. Distribution and ten-year temporal trends (2009–2018) of perfluoroalkyl substances in gull eggs from Spanish breeding colonies. Environ. Pollut. 293, 118555 https://doi.org/10.1016/j.envpol.2021.118555.

Conder, J.M., Hoke, R.A., Wolf, W. de, Russell, M.H., Buck, R.C., 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent Lipophilic compounds. Environ. Sci. Technol. 42, 995–1003. https://doi.org/10.1021/es070895g.

Stockholm Convention, 2022a. Chemicals proposed for listing under the Convention. http://chm.pops.int/theconvention/thepops/chemicalsproposedforlisting/tabid/2510/default.aspx.

Stockholm Convention, 2022b. All POPs listed in the Stockholm Convention. http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx.

Costantino, M., Giovannini, A., Pietroluongo, G., Saintignan, S., Zampollo, A., Giacoma, C., Azzolin, M., 2019. Striped dolphin home ranges within the IMMA and Natura 2000 area "Gulf of Corinth"(Greece) showing an increasing in the number of individuals. In: International Workshop on Metrology for the Sea. Genoa, Italy.

Cousins, I.T., Johansson, J.H., Salter, M.E., Sha, B., Scheringer, M., 2022. Outside the Safe operating Space of a new planetary boundary for per- and polyfluoroalkyl substances (PFAS). Environ. Sci. Technol. https://doi.org/10.1021/acs.est.2c02765.

DeWitt, J.C., Peden-Adams, M.M., Keller, J.M., Germolec, D.R., 2012. Immunotoxicity of perfluorinated compounds: recent developments. Toxicol. Pathol. 40, 300–311. https://doi.org/10.1177/0192623311428473.

Diao, J., Chen, Z., Wang, T., Su, C., Sun, Q., Guo, Y., Zheng, Z., Wang, L., Li, P., Liu, W., Hong, S., Khim, J.S., 2022. Perfluoroalkyl substances in marine food webs from South China Sea: trophic transfer and human exposure implication. J. Hazard Mater. 431 https://doi.org/10.1016/j.jhazmat.2022.128602.

Dorneles, P.R., Lailson-Brito, J., Azevedo, A.F., Meyer, J., Vidal, L.G., Fragoso, A.B., Torres, J.P., Malm, O., Blust, R., Das, K., 2008. High accumulation of perfluorooctane sulfonate (PFOS) in marine tucuxi dolphins (*Sotalia guianensis*) from the Brazilian coast. Environ. Sci. Technol. 42, 5368–5373. https://doi.org/10.1021/es800702k.

Du, D., Lu, Y., Zhou, Y., Li, Q., Zhang, M., Han, G., Cui, H., Jeppesen, E., 2021. Bioaccumulation, trophic transfer and biomagnification of perfluoroalkyl acids (PFAAs) in the marine food web of the South China Sea. J. Hazard Mater. 405 https://doi.org/10.1016/j.jhazmat.2020.124681.

ECHA, E.C.A., 2013. Candidate list of substances of very high concern for authorisation. http://echa.europa.eu/web/guest/candidate-list-table.

Escoruela, J., Garreta, E., Ramos, R., González-Solís, J., Lacorte, S., 2018. Occurrence of per- and polyfluoroalkyl substances in Calonectris shearwaters breeding along the Mediterranean and Atlantic colonies. Mar. Pollut. Bull. 131, 335–340. https://doi.org/10.1016/j.marpolbul.2018.04.032.

European Union (EU), 2013. Directives of 12 August 2013 Amending Directives 2000/60/EC and 2008/105/EC as Regards Priority Substances in the Field of Water Policy.

European Union (EU), 2014. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 32 on Biota Monitoring. https://circabc.europa.eu/sd/a/62343f10-5759-4e7c-ae2b-12677aa57605/Guidance%20No%2032%20-%20Biota%20Monitoring.pdf.

Evich, M.G., Davis, M.J.B., McCord, J.P., Acrey, B., Awkerman, J.A., Knappe, D.R.U., Lindstrom, A.B., Speth, T.F., Tebes-Stevens, C., Strynar, M.J., Wang, Z., Weber, E.J., Henderson, W.M., Washington, J.W., 2022. Per- and polyfluoroalkyl substances in the environment. Science 375. https://doi.org/10.1126/science.abg9065, 1979.

Galatius, A., Dietz, R., Rigét, F.F., Sonne, C., Kinze, C.C., Lockyer, C., Bossi, R., 2011. Temporal and life history related trends of perfluorochemicals in harbor porpoises from the Danish North Sea. Mar. Pollut. Bull. 62, 1476–1483. https://doi.org/10.1016/j.marpolbul.2011.04.011.

Giesy, J.P., Kannan, K., 2001. Global distribution of perfluorooctane sulfonate in wildlife. Environ. Sci. Technol. 35, 1339–1342. https://doi.org/10.1021/es001834k.

Giesy, J.P., Kannan, K., 2002. Perfluorochemical surfactants in the environment. Environ. Sci. Technol. 36, 146A–152A. https://doi.org/10.1021/es022253t.

Goeritz, I., Falk, S., Stahl, T., Schäfers, C., Schlechtriem, C., 2013. Biomagnification and tissue distribution of perfluoroalkyl substances (PFASs) in market-size rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 32, 2078–2088. https://doi.org/10.1002/etc.2279.

Gómez, C., Vicente, J., Echavarri-Erasun, B., Porte, C., Lacorte, S., 2011. Occurrence of perfluorinated compounds in water, sediment and mussels from the Cantabrian Sea (North Spain). Mar. Pollut. Bull. 62, 948–955. https://doi.org/10.1016/j.marpolbul.2011.02.049.

Gui, D., Zhang, M., Zhang, T., Zhang, B., Lin, W., Sun, X., Yu, X., Liu, W., Wu, Y., 2019. Bioaccumulation behavior and spatiotemporal trends of per- and polyfluoroalkyl substances in Indo-Pacific humpback dolphins from the Pearl River Estuary, China. Sci. Total Environ. 658, 1029–1038. https://doi.org/10.1016/j.scitotenv.2018.12.278.

Hart, K., Kannan, K., Isobe, T., Takahashi, S., Yamada, T.K., Miyazaki, N., Tanabe, S., 2008. Time trends and transplacental transfer of perfluorinated compounds in melon-headed whales stranded along the Japanese coast in 1982, 2001/2002, and 2006. Environ. Sci. Technol. 42, 7132–7137. https://doi.org/10.1021/es801224v.

Haug, L.S., Thomsen, C., Brantsæter, A.L., Kvalem, H.E., Haugen, M., Becher, G., Alexander, J., Meltzer, H.M., Knutsen, H.K., 2010. Diet and particularly seafood are major sources of perfluorinated compounds in humans. Environ. Int. 36, 772–778. https://doi.org/10.1016/j.envint.2010.05.016.

Houde, M., Bujas, T.A.D., Small, J., Wells, R.S., Fair, P.A., Bossart, G.D., Solomon, K.R., Muir, D.C.G., 2006. Biomagnification of perfluoroalkyl compounds in the bottlenose dolphin (*Tursiops truncatus*) food web. Environ. Sci. Technol. 40, 4138–4144. https://doi.org/10.1021/es060233b.

Houde, M., De Silva, A.O., Muir, D.C.G., Letcher, R.J., 2011. Monitoring of perfluorinated compounds in aquatic biota: an updated review. Environ. Sci. Technol. 45, 7962–7973. https://doi.org/10.1021/es104326w.

Huang, K., Li, Y., Bu, D., Fu, Jie, Wang, M., Zhou, W., Gu, L., Fu, Y., Cong, Z., Hu, B., Fu, Jianjie, Zhang, A., Jiang, G., 2022. Trophic magnification of short-chain per- and polyfluoroalkyl substances in a terrestrial food chain from the Tibetan plateau. Environ. Sci. Technol. Lett. 9, 147–152. https://doi.org/10.1021/acs.estlett.1c01009.

Ishibashi, H., Iwata, H., Kim, E.Y., Tao, L., Kannan, K., Amano, M., Miyazaki, N., Tanabe, S., Batoev, V.B., Petrov, E.A., 2008. Contamination and effects of perfluorochemicals in baikal seal (*Pusa sibirica*). 1. Residue level, tissue distribution, and temporal trend. Environ. Sci. Technol. 42, 2295–2301. https://doi.org/10.1021/es072054f.

Kannan, K., 2011. Perfluoroalkyl and polyfluoroalkyl substances: current and future perspectives. Environ. Chem. 8, 333. https://doi.org/10.1071/EN11053.

Kannan, K., Koistinen, J., Beckmen, K., Evans, T., Gorzelany, J.F., Hansen, K.J., Jones, P.D., Helle, E., Nyman, M., Giesy, J.P., 2001. Accumulation of perfluorooctane sulfonate in marine mammals. Environ. Sci. Technol. 35, 1593–1598. https://doi.org/10.1021/es001873w.

Kannan, K., Corsolini, S., Falandysz, J., Oehme, G., Focardi, S., Giesy, J.P., 2002. Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean Seas. Environ. Sci. Technol. 36, 3210–3216. https://doi.org/10.1021/es020519q.

Kannan, K., Tao, L., Sinclair, E., Pastva, S.D., Jude, D.J., Giesy, J.P., 2005. Perfluorinated compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. Arch. Environ. Contam. Toxicol. 48, 559–566. https://doi.org/10.1007/s00244-004-0133-x.

Kannan, K., Perrotta, E., Thomas, N.J., 2006. Association between perfluorinated compounds and pathological conditions in southern sea otters. Environ. Sci. Technol. 40, 4943–4948. https://doi.org/10.1021/es060932o.

Kelly, B.C., Ikonomou, M.G., Blair, J.D., Surridge, B., Hoover, D., Grace, R., Gobas, F.A.P. C., 2009. Perfluoroalkyl contaminants in an arctic marine food web: trophic magnification and wildlife exposure. Environ. Sci. Technol. 43, 4037–4043. https://doi.org/10.1021/es9003894.

Khan, B., Burgess, R.M., Cantwell, M.G., 2023. Occurrence and bioaccumulation patterns of per- and polyfluoroalkyl substances (PFAS) in the marine environment. ACS ES&T Water 3, 1243–1259. https://doi.org/10.1021/acsestwater.2c00296.

Kudo, N., Suzuki-Nakajima, E., Mitsumoto, A., Kawashima, Y., 2006. Responses of the liver to perfluorinated fatty acids with different carbon chain length in male and female Mice:in relation to induction of Hepatomegaly, Peroxisomal .BETA.-Oxidation and Microsomal 1-Acylglycerophosphocholine Acyltransferase. Biol. Pharm. Bull. 29, 1952–1957. https://doi.org/10.1248/bpb.29.1952.

Kurwadkar, S., Dane, J., Kanel, S.R., Nadagouda, M.N., Cawdrey, R.W., Ambade, B., Struckhoff, G.C., Wilkin, R., 2022. Per- and polyfluoroalkyl substances in water and wastewater: a critical review of their global occurrence and distribution. Sci. Total Environ. 809, 151003 https://doi.org/10.1016/j.scitotenv.2021.151003.

Kwadijk, C.J.A.F., Korytár, P., Koelmans, A.A., 2010. Distribution of perfluorinated compounds in aquatic systems in The Netherlands. Environ. Sci. Technol. 44, 3746–3751. https://doi.org/10.1021/es100485e.

Lam, J.C.W., Lyu, J., Kwok, K.Y., Lam, P.K.S., 2016. Perfluoroalkyl substances (PFASs) in marine mammals from the South China Sea and their temporal changes 2002-2014: concern for alternatives of PFOS? Environ. Sci. Technol. 50, 6728–6736. https://doi.org/10.1021/acs.est.5b06076.

Lau, C., Thibodeaux, J.R., Hanson, R.G., Rogers, J.M., Grey, B.E., Stanton, M.E., Butenhoff, J.L., Stevenson, L.A., 2003. Exposure to perfluorooctane sulfonate during Pregnancy in rat and Mouse. II: Postnatal evaluation. Toxicol. Sci. 74, 382–392. https://doi.org/10.1093/toxsci/kfg122.

Letcher, R.J., Chu, S., McKinney, M.A., Tomy, G.T., Sonne, C., Dietz, R., 2014. Comparative hepatic in vitro depletion and metaboliteformation of major perfluorooctane sulfonate precursors in Arcticpolar bear, beluga whale, and ringed seal. Chemosphere 112, 225–231.

Li, F., Duan, J., Tian, S., Ji, H., Zhu, Y., Wei, Z., Zhao, D., 2020. Short-chain per- and polyfluoroalkyl substances in aquatic systems: occurrence, impacts and treatment. Chem. Eng. J. 380, 122506 https://doi.org/10.1016/j.cej.2019.122506.

López-Berenguer, G., Bossi, R., Eulaers, I., Dietz, R., Peñalver, J., Schulz, R., Zubrod, J., Sonne, C., Martínez-López, E., 2020. Stranded cetaceans warn of high perfluoroalkyl substance pollution in the western Mediterranean Sea. Environ. Pollut. 267 https://doi.org/10.1016/j.envpol.2020.115367.

Lorenzo, M., Campo, J., Farré, M., Pérez, F., Picó, Y., Barceló, D., 2016. Perfluoroalkyl substances in the Ebro and Guadalquivir river basins (Spain). Sci. Total Environ. 540, 191–199. https://doi.org/10.1016/j.scitotenv.2015.07.045.

Ma, J., Zhu, H., Kannan, K., 2020. Fecal excretion of perfluoroalkyl and polyfluoroalkyl substances in Pets from New York state, United States. Environ. Sci. Technol. Lett. 7, 135–142. https://doi.org/10.1021/acs.estlett.9b00786.

MacLeod, M., Breitholtz, M., Cousins, I.T., Wit, C.A. de, Persson, L.M., Rudén, C., McLachlan, M.S., 2014. Identifying chemicals that are planetary boundary threats. Environ. Sci. Technol. 48, 11057–11063. https://doi.org/10.1021/es501893m.

Martin, J.W., Whittle, D.M., Muir, D.C.G., Mabury, S.A., 2004. Perfluoroalkyl contaminants in a food web from Lake Ontario. Environ. Sci. Technol. 38, 5379–5385. https://doi.org/10.1021/es049331s.

McLachlan, M.S., Holmström, K.E., Reth, M., Berger, U., 2007. Riverine discharge of perfluorinated Carboxylates from the European continent. Environ. Sci. Technol. 41, 7260–7265. https://doi.org/10.1021/es071471p.

Mollenhauer, M.A.M., Carter, B.J., Peden-Adams, M.M., Bossart, G.D., Fair, P.A., 2009. Gene expression changes in bottlenose dolphin, *Tursiops truncatus*, skin cells following exposure to methylmercury (MeHg) or perfluorooctane sulfonate (PFOS). Aquat. Toxicol. 91, 10–18. https://doi.org/10.1016/j.aquatox.2008.09.013.

Munschy, C., Bely, N., Pollono, C., Aminot, Y., 2019. Perfluoroalkyl substances (PFASs) in the marine environment: spatial distribution and temporal profile shifts in shellfish from French coasts. Chemosphere 228, 640–648. https://doi.org/10.1016/j.chemosphere.2019.04.205.

Otero-Sabio, C., Giacomello, M., Centelleghe, C., Caicci, F., Bonato, M., Venerando, A., Graïc, J.-M., Mazzariol, S., Finos, L., Corain, L., Peruffo, A., 2022. Cell cycle alterations due to perfluoroalkyl substances PFOS, PFOA, PFBS, PFBA and the new PFAS C6O4 on bottlenose dolphin (*Tursiops truncatus*) skin cell. Ecotoxicol. Environ. Saf. 244, 113980 https://doi.org/10.1016/j.ecoenv.2022.113980.

Parsons, J.R., Sáez, M., Dolfing, J., de Voogt, P., 2008. Biodegradation of perfluorinated compounds, 53–71. https://doi.org/10.1007/978-0-387-78444-1_2.

Paul, A.G., Jones, K.C., Sweetman, A.J., 2009. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. Environ. Sci. Technol. 43, 386–392. https://doi.org/10.1021/es802216n.

Penland, T.N., Cope, W.G., Kwak, T.J., Strynar, M.J., Grieshaber, C.A., Heise, R.J., Sessions, F.W., 2020. Trophodynamics of per-and polyfluoroalkyl substances in the food web of a large Atlantic Slope river. Environ. Sci. Technol. 54, 6800–6811. https://doi.org/10.1021/acs.est.9b05007.

Pignotti, E., Casas, G., Llorca, M., Tellbüscher, A., Almeida, D., Dinelli, E., Farré, M., Barceló, D., 2017. Seasonal variations in the occurrence of perfluoroalkyl substances in water, sediment and fish samples from Ebro Delta (Catalonia, Spain). Sci. Total Environ. 607–608, 933–943. https://doi.org/10.1016/j.scitotenv.2017.07.025.

Podder, A., Sadmani, A.H.M.A., Reinhart, D., Chang, N.-B., Goel, R., 2021. Per and poly-fluoroalkyl substances (PFAS) as a contaminant of emerging concern in surface water: a transboundary review of their occurrences and toxicity effects. J. Hazard Mater. 419, 126361 https://doi.org/10.1016/j.jhazmat.2021.126361.

Quinete, N., Wu, Q., Zhang, T., Yun, S.H., Moreira, I., Kannan, K., 2009. Specific profiles of perfluorinated compounds in surface and drinking waters and accumulation in mussels, fish, and dolphins from southeastern Brazil. Chemosphere 77, 863–869. https://doi.org/10.1016/j.chemosphere.2009.07.079.

R Core Team, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Rayne, S., Forest, K., 2009. Perfluoroalkyl sulfonic and carboxylic acids: a critical review of physicochemical properties, levels and patterns in waters and wastewaters, and treatment methods. Journal of Environmental Science and Health, Part A 44, 1145–1199. https://doi.org/10.1080/10934520903139811.

Renner, R., 2006. The long and the short of perfluorinated replacements. Environ. Sci. Technol. 40, 12–13. https://doi.org/10.1021/es062612a.

Ritter, S.K., 2010. Fluorochemicals go short. Chemical & Engineering News Archive 88, 12–17. https://doi.org/10.1021/cen-v088n005.p012.

Roscales, J.L., Suárez de Puga, B.R., Vicente, A., Muñoz-Arnanz, J., Sánchez, A.I., Ros, M., Jiménez, B., 2022. Levels and trends of perfluoroalkyl acids (PFAAs) in water (2013–2020) and fish from selected riverine basins in Spain. Chemosphere 286, 131940. https://doi.org/10.1016/j.chemosphere.2021.131940.

Rotander, A., Kärrman, A., Bavel, B. van, Polder, A., Rigét, F., Audunsson, G.A., Víkingsson, G., Gabrielsen, G.W., Bloch, D., Dam, M., 2012. Increasing levels of long-chain perfluorocarboxylic acids (PFCAs) in Arctic and North Atlantic marine mammals. Chemosphere 86, 278–285.. https://doi.org/10.1016/j.chemosphere.2011.09.054.

Saavedra, C., García-Polo, M., Giménez, J., Mons, J.L., Castillo, J.J., Fernández-Maldonado, C., de Stephanis, R., Pierce, G.J., Santos, M.B., 2022. Diet of striped dolphins (*Stenella coeruleoalba*) in southern Spanish waters. Mar. Mamm. Sci. 38, 1566–1582. https://doi.org/10.1111/mms.12945.

Schiavone, A., Corsolini, S., Kannan, K., Tao, L., Trivelpiece, W., Torres, D., Focardi, S., 2009. Perfluorinated contaminants in Fur seal pups and penguin eggs from South Shetland, Antarctica. Sci. Total Environ. 407, 3899–3904. https://doi.org/10.1016/j.scitotenv.2008.12.058.

Schulz, K., Silva, M.R., Klaper, R., 2020. Distribution and effects of branched versus linear isomers of PFOA, PFOS, and PFHxS: a review of recent literature. Sci. Total Environ. 733, 139186 https://doi.org/10.1016/j.scitotenv.2020.139186.

Sciancalepore, G., Pietroluongo, G., Centelleghe, C., Milan, M., Bonato, M., Corazzola, G., Mazzariol, S., 2021. Evaluation of per- and poly-fluorinated alkyl substances (PFAS) in livers of bottlenose dolphins (*Tursiops truncatus*) found stranded along the northern Adriatic Sea. Environ. Pollut. 291 https://doi.org/10.1016/j.envpol.2021.118186.

Sharpe, R.L., Benskin, J.P., Laarman, A.H., MacLeod, S.L., Martin, J.W., Wong, C.S., Goss, G.G., 2010. Perfluorooctane sulfonate toxicity, isomer-specific accumulation, and maternal transfer in zebrafish (Danio rerio) and rainbow trout (Oncorhynchus mykiss). Environ Toxicol Chem n/a-n/a. https://doi.org/10.1002/etc.257.

Stockin, K.A., Yi, S., Northcott, G.L., Betty, E.L., Machovsky-Capuska, G.E., Jones, B., Perrott, M.R., Law, R.J., Rumsby, A., Thelen, M.A., Graham, L., Palmer, E.I., Tremblay, L.A., 2021. Per- and polyfluoroalkyl substances (PFAS), trace elements and life history parameters of mass-stranded common dolphins (*Delphinus delphis*) in New Zealand. Mar. Pollut. Bull. 173 https://doi.org/10.1016/j.marpolbul.2021.112896.

Sun, J., Bossi, R., Bustnes, J.O., Helander, B., Boertmann, D., Dietz, R., Herzke, D., Jaspers, V.L.B., Labansen, A.L., Lepoint, G., Schulz, R., Sonne, C., Thorup, K., Tøttrup, A.P., Zubrod, J.P., Eens, M., Eulaers, I., 2019. White-tailed eagle (*Haliaeetus albicilla*) body feathers document spatiotemporal trends of perfluoroalkyl substances in the northern environment. Environ. Sci. Technol. 53, 12744–12753. https://doi.org/10.1021/acs.est.9b03514.

Tomy, G.T., Budakowski, W., Halldorson, T., Helm, P.A., Stern, G.A., Friesen, K., Pepper, K., Tittlemier, S.A., Fisk, A.T., 2004. Fluorinated organic compounds in an Eastern arctic marine food web. Environ. Sci. Technol. 38, 6475–6481. https://doi.org/10.1021/es049620g.

UNEP, 2006. Risk Profile on Perfluorooctane Sulfonate. Geneva.

UNEP, 2009. Governments Unite to Step-Up Reduction on Global DDT Reliance and Add Nine New Chemicals under International Treaty. http://chm.pops.int/Convention/Pressrelease/COP4Geneva8May2009/tabid/542/language/en-US/Default.aspx.

UNEP, 2017. Risk Management Evaluation on Pentadecafluorooctanoic Acid (CAS No: 335-67-1, PFOA, Perfluorooctanoic Acid), its Salts and PFOA-Related Compounds. Rome.

UNEP, 2019. Risk Management Evaluation on Perfluorohexane Sulfonic Acid (PFHxS), its Salts and PFHxS-Related Compounds. Rome.

UNEP, 2022. Risk Profile for Long-Chain Perfluorocarboxylic Acids, Their Salts and Related Compounds. Rome.

US EPA, 2006. 2010/15 PFOA Stewardship Program.

van de Vijver, K.I., Hoff, P.T., Das, K., van Dongen, W., Esmans, E.L., Jauniaux, T., Bouquegneau, J.M., Blust, R., de Coen, W., 2003. Perfluorinated chemicals infiltrate ocean waters: Link between exposure levels and stable isotope ratios in marine mammals. Environ. Sci. Technol. 37, 5545–5550. https://doi.org/10.1021/es0345975.

Wang, Z., Cousins, I.T., Scheringer, M., Buck, R.C., Hungerbühler, K., 2014. Global emission inventories for C4–C14 perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and emissions from quantifiable sources. Environ. Int. 70, 62–75. https://doi.org/10.1016/j.envint.2014.04.013.

Wang, Z., Boucher, J.M., Scheringer, M., Cousins, I.T., Hungerbühler, K., 2017. Toward a comprehensive global emission inventory of $C_4$–$C_{10}$ Perfluoroalkanesulfonic acids (PFSAs) and related precursors: focus on the life cycle of $C_8$-based products and ongoing industrial transition. Environ. Sci. Technol. 51, 4482–4493. https://doi.org/10.1021/acs.est.6b06191.

Yeung, L.W.Y., Dassuncao, C., Mabury, S., Sunderland, E.M., Zhang, X., Lohmann, R., 2017. Vertical profiles, sources, and transport of PFASs in the arctic ocean. Environ. Sci. Technol. 51, 6735–6744. https://doi.org/10.1021/acs.est.7b00788.

Zhang, T., Sun, H., Qin, X., Gan, Z., Kannan, K., 2015. PFOS and PFOA in paired urine and blood from general adults and pregnant women: assessment of urinary elimination. Environ. Sci. Pollut. Control Ser. 22, 5572–5579. https://doi.org/10.1007/s11356-014-3725-7.

Zhang, X., Zhang, Y., Dassuncao, C., Lohmann, R., Sunderland, E.M., 2017. North Atlantic Deep Water formation inhibits high Arctic contamination by continental perfluorooctane sulfonate discharges. Global Biogeochem. Cycles 31, 1332–1343. https://doi.org/10.1002/2017GB005624.