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PFAS profiles in three North Sea top predators: metabolic differences among species?

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Abstract Profiles of seven compounds of perfluoro-alkyl substances (PFASs) were compared among three species of top predators from the Danish North Sea: the white-beaked dolphin (*Lagenorhynchus albirostris*), the harbor porpoise (*Phocoena phocoena*), and the harbor seal (*Phoca vitulina*). The seals had higher total burdens (757.8 ng g^{-1} ww) than the dolphins (439.9 ng g^{-1} ww) and the porpoises (355.8 ng g^{-1} ww), probably a reflection of feeding closer to the shore and thus contamination sources. The most striking difference among the species was the relative contribution of perfluorooctanesulfonamide (PFOSA) to the profiles; the seals (0.1 %) had much lower levels than porpoises (8.3 %) and dolphins (26.0 %). In combination with the values obtained from the literature, this result indicates that Carnivora species including Pinnipedia have a much higher capacity of transforming PFOSA to perfluorooctane sulfonic acid (PFOS) than cetacean species. Another notable difference among the species was that the two smaller species (seals and porpoises) with supposedly higher metabolic rates had lower concentrations of the perfluorinated carboxylic acids, which are generally more easily excreted than perfluorinated sulfonamides. Species-specific characteristics should be recognized when PFAS contamination in marine mammals is investigated, for example, several previous studies of PFASs in cetaceans have not quantified PFOSA.

Keywords Harbor porpoise · Harbor seal · Metabolism · North Sea · Perfluorinated alkylated substances · White-beaked dolphin

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Introduction

Perfluorinated alkylated substances (PFASs) are synthetic compounds with several applications, e.g., fire-fighting foams, cleaners, lubricants, and various coatings (Kissa 2001). They are persistent in the environment, and although the largest producer in the world, the 3 M Company seized production of perfluorooctane sulfonic acid (PFOS) in 2002 and perfluorooctanoic acid (PFOA) in 2008, these substances continue to be in the environment and bioaccumulate in biota (Giesy and Kannan 2001; Kissa 2001; Kannan et al. 2001, 2005; Butt et al. 2007). PFOS is still produced in China, but emission levels are not known (Lim et al. 2011). Two PFASs (PFOS and perfluorooctane sulfonyl fluoride (PFOS-F) have been listed under the Stockholm Convention on persistent organic pollutants (POPs) (Wang et al. 2009). Among the reported effects of PFASs are reproductive toxicity (Lau et al. 2003; Luebker et al. 2005), neurotoxicity (Johansson et al. 2008; Liu et al. 2010), hepatotoxicity (Miller et al. 1975; Malinverno et al. 2005), immunotoxicity (Grandjean et al. 2012), and effects on metabolism (Berthiaume and Wallace 2002). Unlike most other POPs, which accumulate in lipid-

rich tissue, PFASs bind to blood proteins and accumulate mainly in the liver, the kidneys, and bile secretion (Jones et al. 2003). Given the persistence and bioaccumulation potential of PFASs, their toxicity to wildlife at high trophic levels is of concern. Marine mammals occupy the highest trophic levels in the marine food web, and high concentrations have been reported in several species at diverse locations (e.g., Van de Vijver et al. 2004; Dietz et al. 2008; Dorneles et al. 2008; Butt et al. 2010).

It is widely accepted that PFOS is the predominant PFAS in wildlife samples (Martin et al. 2004; Houde et al. 2006). While this is also the case for many cetacean species, a literature survey revealed several studies reporting cetacean species carrying perfluorooctanesulfonamide acid (PFOSA) concentrations similar to or greater than PFOS concentrations. In bottlenose dolphins (*Tursiops truncatus*) a PFOS/FOSA ratio of 0.6 has been reported, in common dolphin (*Delphinus delphis*) a ratio of 1.1, in melon-headed whale (*Peponocephala electra*) 0.9, in narwhal (*Monodon monoceros*) 1.8, in beluga (*Delphinapterus leucas*) different studies have reported ratios of 0.1–1.0, in long-finned pilot whale (*Globicephala melas*) ratios of 0.7–5.4, and in minke whale (*Balaenoptera acutorostrata*) ratios of 0.2–12.3 (Kannan et al. 2002; Muir et al. 2004; Tomy et al. 2004a; Bossi et al. 2005a; Hart et al. 2008; Moon et al. 2010; Reiner et al. 2011). On the other hand, marine mammals from the order Carnivora (including Pinnipedia) have consistently shown much greater concentrations of PFOS than PFOSA as seen in, e.g., polar bear (*Ursus maritimus*, ratios 241.7–1552.9), harbor seal (*Phoca vitulina*, ratios 76.2–116.9), and ringed seal (*Pusa hispida*, ratios 9.5–336.0) (Martin et al. 2004; Smithwick et al. 2005; Bossi et al. 2005b; Butt et al. 2008; Dietz et al. 2008, 2012; Shaw et al. 2009; Ahrens et al. 2009). However, all these studies represent different geographical areas and timespans, so the recorded PFAS profiles are not directly comparable between the phylogenetic groups.

PFAS toxicity is dependent on the specific profile as well as on the total burden. Longer chain compounds with a sulfonate group appear to exhibit stronger biological effects than short chain compounds with a carboxylate group (Liao et al. 2009). Although relatively few studies have focused on toxicity of PFOSA, it seems to differ from PFOS in terms of toxicity. PFOSA has shown stronger neurotoxicity than PFOS, possibly due to its increased hydrophobicity (Slotkin et al. 2008). Although PFOSA is often the predominant PFAS in cetacean samples, several studies of PFAS in cetaceans have not quantified this substance (e.g., Van de Vijver et al. 2003, 2007; Dorneles et al. 2008; Law et al. 2008).

In the current study, we aimed to minimize time and space as sources of variation by focusing on PFAS profiles of three marine mammal species from the same area—harbor porpoise (*Phocoena phocoena*), white-beaked dolphin (*Lagenorhynchus albirostris*), and harbor seal from the North Sea—with samples from the same time period (1999–2002). In the North Sea, marine mammals show high PFAS concentrations (Law et al. 2008; Ahrens et al. 2009; Galatius et al. 2011; Dietz et al. 2012). We aimed to interpret observed differences in relation to excretion rates or metabolism of PFASs and published studies of prey preferences.

Materials and methods

The samples

The harbor seal sample consisted of 13 individuals collected at haul-outs in the Wadden Sea during the phocine distemper virus outbreak in the summer of 2002 (Härkönen et al. 2006). Data from these individuals made up part of the sample for Dietz et al. (2012). The harbor porpoise sample consisted of 11 individuals collected from by-catches in the Danish North Sea in the years 1999–2002. Data from these individuals made up part of the sample for Galatius et al. (2011). The white-beaked dolphin (*L. albirostris*) sample consisted of seven specimens stranded on the Danish North Sea coast during the years 1999–2002 (Kinze et al. 2011), and collected in cooperation with the Natural History Museum, University of Copenhagen. Due to the life history-related differences in PFAS profiles and concentrations of harbor porpoises observed by Galatius et al. (2011), no neonates, suckling juveniles, or lactating females were included for any species in this study. Harbor seals and harbor porpoises were classified according to age information from the decalcified teeth (Dietz et al. 1991; Hohn and Lockyer 1995; Lockyer et al. 2010), while the white-beaked dolphins were aged based on their size (Galatius et al. 2012).

Extraction and analysis

Liver samples were frozen and stored at −20 °C. In the laboratory, samples were lightly thawed and homogenized before chemical analysis. Seven compounds were quantified: PFOS, PFOSA, perfluorohexane sulfonate (PFHxS), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnA). The extraction method was based on ion pairing as described by Bossi et al. (2005b). $^{13}C_2$-PFDA and $^{13}C_4$-PFOS were used as surrogate standards. Instrumental analysis was performed by liquid chromatography–tandem mass spectrometry with electrospray ionization. The extracts (20 μL injection volume) were chromatographed on a C18 Betasil column (2.1, 50 mm, Thermo Hypersil-Keystone, Bellefonte, PA, USA) using an Agilent 1100 Series HPLC (Agilent Technologies, Palo Alto, CA, USA). The HPLC was interfaced to a triple quadrupole API 2000 (Sciex, Concord, Ontario, Canada)

equipped with a TurboIon Spray source operating in negative ion mode. Detection limits ranged from 0.2 to 1.4 ng g^{-1} ww. Instrument set-up, quality assurance, and calibration procedures, as well as the standards and reagents used are described in detail by Bossi et al. (2005b).

To investigate the relative contributions of the PFASs and their covariance pattern in relation to geography, a principal components analysis (PCA) was performed on the covariance matrix of the concentration data. To standardize the impact of each compound on the analysis, variance was normalized to unit. The statistical analyses were performed using the statistical packages in R (R Development Core Team 2008).

Results and discussion

The three species showed considerable variation, both in terms of PFAS concentrations and profiles (Table 1, Fig. 1). Some of the observed variation is probably related to differences in prey preference and, thus, differences in specific intake of the analyzed compounds. Harbor porpoises have been found to eat mainly demersal fish species. In the North Sea, Aarefjord et al. (1995) found the most important species (by weight) to be whiting (ca 40 %, *Merlangius merlangus*), cod (ca 25 %, *Gadus morhua*), and eel pout (ca 15 %, *Zoarces viviparus*). Harbor seals eat mainly demersal fish species of which Hall et al. (1998) found the most important (by weight) to be whiting (24 %), sole (15 %, *Solea solea*), dragonet (13 %, *Callionymus lyra*), and sand goby (11 %, *Pomatoschistus minutus*). White-beaked dolphins seem to be specializing in Gadidae species (Jansen et al. 2010). These authors found cod (56 %) and whiting (38 %) to be the dominant species (by weight) in the diet of white-beaked dolphins from the North Sea. We have not been able to find relevant data to assess the intake of different PFASs from these prey species in the North Sea. However, these diet studies reveal large overlaps, indicating that other factors are more important as explanations for the observed differences.

Harbor seals showed the highest concentrations of ∑PFAS (757.8 ng g^{-1} ww, SD 246.8) followed by white-beaked dolphins (439.9 ng g^{-1} ww, SD 202.6) and harbor porpoises (355.8 ng g^{-1} ww, SD 153.7). The higher concentrations in the seals relative to the cetaceans may be related to different metabolisms but may also be accounted for by harbor seals feeding close to shore (Tougaard et al. 2006; Herr et al. 2009) and thus closer to contamination sources.

Perfluorosulfonic acids

In terms of the perfluorosulfonic acid (PFSA) profiles, the most striking difference was the contribution of PFOSA, which was very low in the harbor seals (0.1 % of ∑PFAS), intermediate in the harbor porpoises (8.3 %), and high in the

Table 1 Basic data and PFAS liver concentration data (ng g^{-1} ww) from the harbor seals, harbor porpoises, and white-beaked dolphins from the North Sea, collected in 1999–2002, including mean, mean contribution (%), standard deviation (SD), median (Med), and range

		Harbor seal (*N*=13)	Harbor porpoise (*N*=11)	White-beaked dolphin (*N*=7)
PFOS	Mean	689.1	325.3	289.6
	%	93.2	89.1	64.8
	SD	236.2	153.6	158.4
	Med	634.0	391.0	229.0
	Min	430.0	89.0	126.0
	Max	1284.2	534.0	540.0
PFOSA	Mean	0.9	16.0	122.0
	%	0.1	6.7	28.5
	SD	0.7	9.5	104.9
	Med	0.7	12.3	104.0
	Min	ND	7.0	4.3
	Max	2.9	32.1	283.0
PFHxS	Mean	16.3	1.1	2.8
	%	2.1	0.4	0.6
	SD	7.9	1.8	2.6
	Med	14.6	<DL	1.2
	Min	6.5	<DL	<DL
	Max	32.4	6.3	6.8
PFOA	Mean	1.8	<DL	1.1
	%	0.2	0.2	0.2
	SD	1.5	0.0	1.4
	Med	1.8	<DL	<DL
	Min	ND	<DL	<DL
	Max	6.1	<DL	4.4
PFNA	Mean	8.7	<DL	4.2
	%	1.4	0.3	1.0
	SD	3.6	0.0	1.3
	Med	9.0	<DL	3.9
	Min	ND	<DL	2.4
	Max	15.1	<DL	6.3
PFDA	Mean	14.8	4.9	9.7
	%	2.1	1.3	2.2
	SD	6.1	5.1	4.4
	Med	14.7	3.2	7.9
	Min	7.1	<DL	5.2
	Max	28.3	15.0	17.8
PFUnA	Mean	5.1	7.2	10.6
	%	0.9	2.0	2.6
	SD	1.8	7.6	2.7
	Med	5.5	5.2	9.9
	Min	2.0	<DL	7.7
	Max	8.7	29.0	13.6
∑PFAS	Mean	757.8	355.8	439.9
	SD	246.8	153.7	202.6
	Med	720.0	419.2	407.3
	Min	457.7	106.8	265.9
	Max	1,364.7	589.0	868.7

<*DL* below detection limit, *ND* not detected

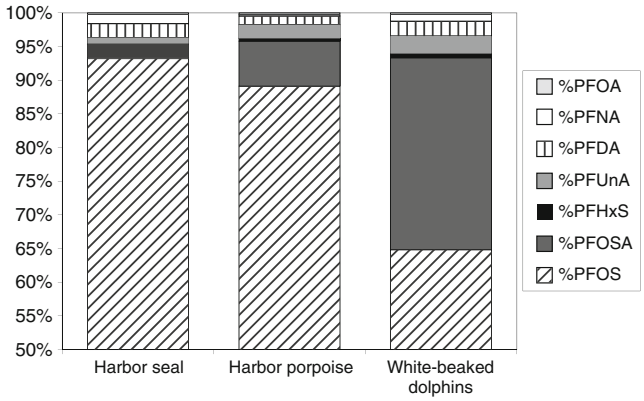

Fig. 1 Plot of the profiles of seven PFASs in liver tissue from harbor seals, harbor porpoises, and white-beaked dolphins from the North Sea

white-beaked dolphins (26.0 %) (Fig. 1). It is probable that these differences reflect basic physiological differences between Carnivora and Cetacea. Biotransformation of PFOSA to PFOS has been demonstrated in vertebrate liver microsomes (Tomy et al. 2004b; Xu et al. 2004). Phylogenetic differences in capacity for transformation are likely, resulting in different, species-specific balances between the two compounds. Although PFOS is usually the predominant PFAS in wildlife matrices (Martin et al. 2004; Houde et al. 2006), concentrations of PFOSA similar to or even higher than PFOS concentrations have also been reported for several cetacean species (e.g., melon-headed whale, bottlenose dolphin, common dolphin, minke whale, and pilot whale) (Hart et al. 2008; Kannan et al. 2002; Bossi et al. 2005a). Except for one example which is the sea otter (*Enhydra lutris*) (Hart et al. 2009), we are not aware of such findings from Carnivora species, where PFOS concentrations are otherwise consistently many times higher than PFOSA concentrations (Bossi et al. 2005b; Dietz et al. 2008; Hart et al. 2009; Dietz et al. 2012 among others, see also Fig. 2). New results show that the PFOS/FOSA ratio in polar bear liver is much higher than that in other tissues, indicating effective transformation of PFOSA to PFOS in the liver of this carnivore (Greaves et al. 2012). PFOSA is also effectively transformed to PFOS in Sprague–Dawley rats (half-life estimates range from 2.5 to 5.9 days, for different isomers) (Ross et al. 2012). Fast transformation of PFOSA to PFOS fits well with the pattern of PFOS/PFOSA ratios among the carnivore species listed in Fig. 2. Polar bears, which have the highest ratios, may go through long periods fasting between meals (Ramsay and Stirling 1988) and will, thus, have transformed the vast majority of ingested PFOSA between meals. On the other hand, the lowest recorded ratio among the carnivores is in the sea otter (Fig. 2), which may be explained by very frequent meals. Sea otters need to

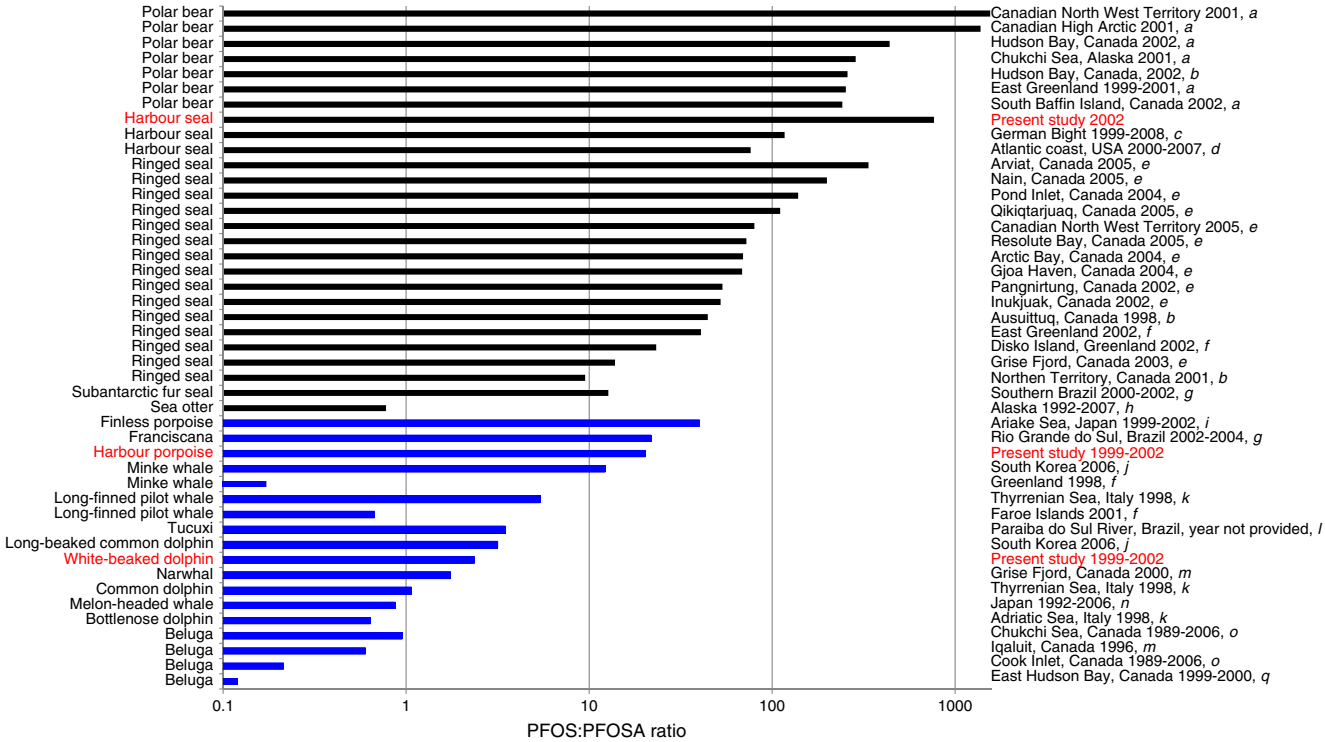

Fig. 2 PFOS/PFOSA ratios in liver tissue of marine mammals from available literature. Carnivores are shown as *black bars*, cetaceans as *blue*. Data points from the present study are specified in *red*. A logarithmic scale is used, making differences apparently smaller. References: *a* Smithwick et al. 2005, *b* Martin et al. 2004, *c* Ahrens et al. 2009, *d* Shaw et al. 2009, *e* Butt et al. 2008, *f* Bossi et al. 2005a, *g* Leonel et al. 2008, *h* Hart et al. 2009, *i* Nakata et al. 2006, *j* Moon et al. 2010, *k* Kannan et al. 2002, *l* Quinete et al. 2009, *m* Tomy et al. 2004a, *n* Hart et al. 2008, *o* Reiner et al. 2011, *q* Muir et al. 2004

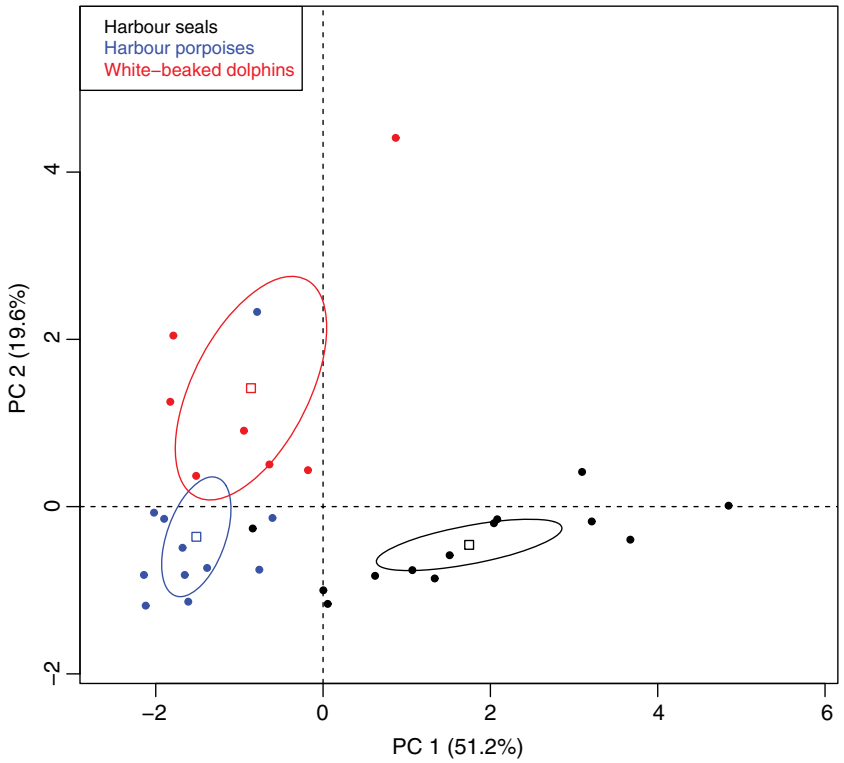

Fig. 3 Scores along PC1 and PC2 from the PCA of PFAS profiles in harbor seals, harbor porpoises, and white-beaked dolphins from the North Sea. *Dots* indicate individual scores, *squares* are centroid positions of the species, and *ellipses* show the 95 % confidence intervals of the centroid positions. Broken lines show the 0 or average values of the PCs

ingest up to a quarter of their own mass in prey items daily (Morrison et al. 1974). Among the cetacean species, this pattern where species with frequent food intake have lower ratios does not occur. Small cetaceans, such as harbor porpoise, finless porpoise, and franciscana, need to feed on a daily basis because of high heat loss and limited capacity to store energy (Koopman 1998). However, these three species have the highest ratios recorded among the cetaceans, indicating a reverse pattern to that seen in carnivores. This is also reflected among our samples, as harbor porpoises have larger ratios than the white-beaked dolphins. This can be explained either by higher capacity for transformation in the smaller cetacean species than the larger, or if the higher metabolism of the smaller cetacean species more than outweighs their more frequent intake of PFOSA. Under either scenario, it reflects very different patterns between cetaceans and carnivores.

Thus, given the present results from species with similar diet from the same area and period, we find that a general phylogenetic difference in the ability to transform PFOSA to PFOS is by far the most probable explanation for the observed differences. As mentioned, the difference between the two cetaceans may be related to different rates of metabolism. In a comparison of PFAS profiles across harbor porpoises at different life history stages, Galatius et al. (2011) found the greatest PFOS/PFOSA ratios in porpoises with perceived increased rates of metabolism, namely neonates, young juveniles, and lactating females. Harbor porpoises are smaller than white-beaked dolphins and have a much more rapid life cycle than white-beaked dolphins (Read and Hohn 1995; Galatius et al. 2013) and, most likely, a higher rate of metabolism.

Another difference between the seals and the cetaceans was the higher concentrations of PFHxS found in the seals. An explanation could be that the relatively short-chained PFHxS (C_6) is more rapidly excreted in cetaceans. Given the apparently more rapid excretion of perfluorinated carboxylic acids (PFCAs) in harbor seals and porpoises relative to white-beaked dolphins (see next page), this does not seem a likely explanation. Other studies of North Sea and Baltic harbor seals have found similar PFHxS concentrations (Ahrens et al. 2009; Dietz et al. 2012), while a study of harbor seals from the East Atlantic (Shaw et al. 2009) recorded much lower concentrations, indicating differences in ingestion rather than excretion as the background for this particular difference.

Table 2 Component loadings of the seven analyzed PFASs on principal components 1 and 2 from the PCA

Compound	PC1 loading	PC2 loading
PFOS	0.89	−0.09
PFOA	0.59	0.37
PFHxS	0.93	−0.16
PFOSA	−0.24	0.81
PFNA	0.87	0.02
PFDA	0.88	0.25
PFUnA	−0.06	0.70

Perfluorinated carboxylic acids

Harbor porpoises and harbor seals showed lower concentrations of perfluorinated carboxylic acids (∑PFCA; 3.8 and 4.6 %, respectively) than white-beaked dolphins (6.0 %). The two shorter-chained PFCAs, PFOA (C_8) and PFNA (C_9), were not detected above detection limit among any of the porpoises, while they were found in all seals and dolphins except one seal. The inter-specific differences of the concentrations of the two longer-chained compounds PFDA (C_{10}) and PFUnA (C_{11}) were smaller than those of the shorter-chained compounds. PFCAs are usually excreted much faster than PFSAs of the same chain length (Stahl et al. 2011), and the two smaller species (porpoises and seals) are likely to have higher metabolic rates and may thus almost eliminate the more easily excreted compounds soon after ingestion. This may also be reflected in the very low contribution of the shortest-chained analyzed PFSA, PFHxS, in the porpoises (0.4 %).

Ordination along components 1 and 2 of the PCA provided almost complete separation of the three species (Fig. 3). PC1 and PC2 combined explained 71 % of the total variance. PC1 showed strong positive loadings on all investigated compounds, except PFOSA and PFUnA with moderate negative and neutral loadings, respectively (Table 2). PC2 showed strong positive loadings on PFOSA and PFUnA, moderate positive loadings on PFOA and PFDA, and more neutral loadings on PFOS, PFHxS, and PFNA (Table 2). Harbor seals were separated from the two cetacean species by higher scores along PC1, while white-beaked dolphins were separated from the other two species by higher scores along PC2. PC1 may be seen as mainly reflecting the phylogenetically dependent ability to transform PFOSA. PC2 may be seen as reflecting differences in metabolic rate, where the more easily excreted compounds (except PFHxS; see previous) show lower concentrations in the two smaller species with higher metabolic rates. In conclusion, our study shows that PFAS profiles may vary considerably among top predators from the same area and underscores the importance of studying a well-chosen array of compounds. Although PFOS is usually the predominant PFAS in wildlife matrices (Martin et al. 2004; Houde et al. 2006), quantification of PFOS without PFOSA is often not a reliable indicator of PFAS load in cetaceans. This seems to be different from Carnivora with regard to biotransformation of PFOSA. The evolutionary split between cetaceans and their terrestrial ancestors occurred more than 50 million years ago (Thewissen and Williams 2002). During the vast majority of their evolutionary history, they have lived exclusively on fish, squid, and invertebrates. Pinnipeds have a fossil record going back 25–27 million years (Berta 2008), while the polar bear has diverged from the other ursids within the last million years (Hailer et al. 2012) and still occasionally eats plants (Clarkson and Stirling 1994). These differences in the evolutionary time that the different marine mammal lineages have lived on exclusively animal-based diets may have had an impact on their ability to metabolize contaminants, as they will not have had to deal with, e.g., plant toxins during that time. This may have led to differences in the enzyme systems responsible for metabolism of xenobiotic substances among the groups. Several studies have previously investigated PFAS contamination in cetaceans without quantifying PFOSA (e.g., Van de Vijver et al. 2003, 2007; Dorneles et al. 2008; Law et al. 2008). This practice can potentially lead to significant underestimates of PFAS burdens.

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