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Feasibility of using the National Marine Mammal Tissue Bank for retrospective exploratory studies of perfluorinated alkyl acids☆

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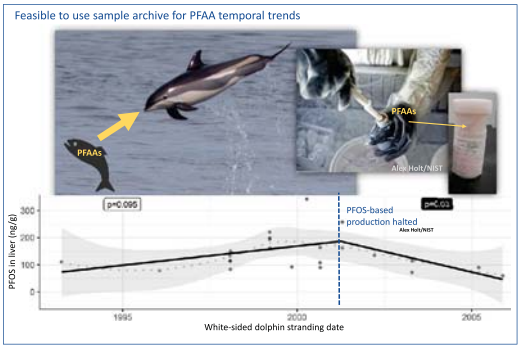
HIGHLIGHTS

- Perfluorinated alkyl acids (PFAAs) can leach into samples during archiving processes.
- PFAAs were measured in the U.S. National Marine Mammal Tissue Bank (NMMTB).
- Leaching of two PFAAs was negligible, while perfluorooctanoate (PFOA) was problematic.
- Southern rough-toothed dolphins had higher PFAAs than northern white-sided dolphins.
- Perfluorooctane sulfonate (PFOS) significantly decreased after 2001 in liver of both species.

GRAPHICAL ABSTRACT



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ABSTRACT

Perfluorinated alkyl acids (PFAAs) have been used for 50+ years in materials such as stain-resistant treatments for paper and clothing, lubricants, and foam fire extinguishers. PFAAs are characterized by a fully fluorinated alkyl chain with a terminal acid group. Their long half-lives and ubiquitous environmental distribution create considerable concern for wildlife and human exposure. There is interest in examining temporal trends of PFAAs using the National Marine Mammal Tissue Bank (NMMTB), but NMMTB tissues are frozen and cryohomogenized in polytetrafluoroethylene (PTFE)-based materials. Because PTFE supplies may leach PFAAs into samples, this study mimicked collection, processing and storage steps of NMMTB samples and measured PFAA leaching to determine the feasibility of using this sample archive for PFAA temporal trends. We also explored concentrations in Atlantic white-sided dolphin (*Lagenorhynchus acutus*, WSDs) and rough-toothed dolphin (*Steno bredanensis*, RTDs) blubber ($n = 3$ and 0) and liver ($n = 48$ and 12, respectively). The materials used in NMMTB protocols may add up to 0.968 ng/g perfluorooctanoic acid (PFOA), 0.090 ng/g perfluorononanoic acid (PNFA), and 0.221 ng/g perfluorooctane sulfonate (PFOS) to each archived sample. Leaching of PFNA and PFOS from supplies compared to dolphin levels was negligible, but PFOA contributions were substantially higher than levels found in most dolphin liver samples. Therefore, monitoring PFOA temporal trends from the NMMTB would require careful consideration. RTDs had significantly higher levels of PFOS and PFNA than WSDs. Both species have similar life history, trophic status, and foraging behaviors in deep pelagic waters, so differences could be from latitudinal

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variation in contamination. RTDs stranded in Florida; WSDs stranded farther north mostly in Massachusetts. Juveniles had significantly higher levels of PFOS and PFNA than adults in both species, suggesting growth dilution as they approach maturity. PFOS significantly decreased after 2001 in both species as expected based on changes in production.

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1. Introduction

Perfluorinated alkyl acids (PFAAs) have been manufactured for >50 years and are used in materials such as stain-resistant treatments for paper and clothing, lubricants, and in foam fire extinguishers. PFAAs are characterized by a fully fluorinated alkyl chain with a terminal acid group. Their long half-lives and ubiquitous environmental distribution create considerable concern for wildlife and human exposure. The two most commonly measured PFAAs are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) with measurements of shorter and longer chain PFAAs becoming more common, including perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUnA). PFAAs appear to be the ultimate degradation products of several commercially used perfluorinated compounds, are globally distributed throughout the environment, and bioaccumulate in blood and liver rather than fatty tissues (Hekster et al., 2003; Schultz et al., 2003).

Temporal studies have shown that PFAA concentrations increased in marine biota from the 1980s to early 2000s (Sturm and Ahrens, 2010). Recent declines in some environmental samples have been noted for PFOS since 3M, a global science company, voluntarily halted production of PFOS-based chemistry in 2002 (Houde et al., 2011; Newsted et al., 2017; O'Connell et al., 2010; Riget et al., 2013; Sturm and Ahrens, 2010). Some of these studies have taken advantage of the benefits of using samples archived in long-term, formal specimen banks. Another specimen bank that can provide samples for exploring PFAAs is the U.S. National Marine Mammal Tissue Bank (NMMTB) initiated in 1989 and maintained by the National Institute of Standards and Technology (NIST). NMMTB tissues are sampled under strict protocols, maintained frozen at −180 °C in polytetrafluoroethylene(PTFE)-based materials, and cryohomogenized in PTFE disc mills under cleanroom conditions (Becker et al., 1999). Because these PTFE materials may leach PFAAs into samples that they touch, great care must be taken during sample collection, processing and analysis if the samples are to be used for PFAA measurements (Begley et al., 2005; Flaherty et al., 2005). Thus, this study began by testing the feasibility of measuring PFAAs in NMMTB samples.

Two species were chosen from the NMMTB for analysis in this study: the Atlantic white-sided dolphin (*Lagenorhynchus acutus*) and rough-toothed dolphin (*Steno bredanensis*). The Atlantic white-sided dolphin (WSD) inhabits cooler waters from Cape Cod to the United Kingdom and the current samples were collected from animals that stranded mainly in Massachusetts, U.S.A., whereas the warmer subtropical and tropical rough-toothed dolphins (RTDs) in this study stranded in Florida. Both species are similar in their pelagic diets of mainly fish and squid and their age and length at maturity (Clarke, 1986; Craddock et al., 2009; Layne, 1965; Sergeant et al., 1980; Waring et al., 2010). WSDs mature between six and 12 years (201–210 cm) for females and seven to 12 years (220 cm) for males, and RTDs mature around 14 years (225 cm) for males and 10 years (210 cm) for females

Table 1
PFAA concentrations in sampling, processing, and storage materials and estimates of PFAA additions (in bold, bottom 3 rows) to NMMTB samples from these materials.

	Concentration in Materials				Estimated surface area or volume touching the sample	Estimated ng transferred to entire sample		
NMMTB materials	PFOS	PFOA	PFNA	units		PFOS	PFOA	PFNA
Materials that touch the initial 300 g sample								
Sampling gloves								
Glove type 1 (vinyl, used 1998–2005)	21.1	13.8	12.9	ng/g	≈4 × 4 inch, one side	6.2	4.0	3.8
Glove type 2 (vinyl, used 2003–2008)	259	178	13.0	ng/g	≈4 × 4 inch, one side	52	35	2.6
Glove type 3 (copolymer, used 2008-present)	<3.42	<3.44	<3.28	ng/g	≈4 × 4 inch, one side	0.52	0.53	0.50
Water from PTFE squirt bottle	0.036	0.046	<0.025	ng/mL	100 mL	3.56	4.60	2.50
FEP bags	5.86	160	0.879	ng/g	≈4 × 4 inch, one side	5.84	159	0.88
Materials that touch the 150 g subsample								
180 mL PTFE jars	<0.0105	<0.0112	<0.0157	ng/g of water	5% of 150 g sample	0.08	0.08	0.12
Cryohomogenization gloves	3.73	1.35	1.16	ng/g	negligible	0	0	0
Cryohomogenized serum samples	2.29	0.796	0.207	ng/mL		–	–	–
Blank serum samples	2.27	0.492	0.142	ng/mL		–	–	–
Addition from cryohomogenization process	0.017	0.304	0.066	ng/mL	150 mL sample	2.50	45.7	9.83
15 mL PTFE jars	<0.00410	<0.00439	<0.00614	ng/mL of water	1 mL	0.00410	0.00439	0.00614
					Estimated ng/g addition to samples from material contamination 1998–2005	***0.069***	***0.864***	***0.090***
					Estimated ng/g addition to samples from material contamination 2003–2008	***0.221***	***0.968***	***0.086***
					Estimated ng/g addition to samples from material contamination 2008-present	***0.050***	***0.852***	***0.079***

FEP = fluorinated ethylene propylene; PTFE = polytetrafluoroethylene.

Table 2
PFAA mass fractions in three paired Atlantic white-sided dolphin liver and blubber samples.

Animal ID	Tissue	PFOS (ng/g)	PFOA (ng/g)	PFNA (ng/g)
MH-98-440La	Liver	159	<0.56	10
	Blubber	14	<0.56	1.1
MH-99-540La	Liver	243	<0.56	8.5
	Blubber	18	<0.56	1.1
MH-00-724La	Liver	144	0.705	11
	Blubber	22	<0.56	2.3

(Miyazaki and Perrin, 1994; Sergeant et al., 1980). Both species have been understudied for PFAA exposure; in fact only two samples of each species have ever been analyzed for PFAAs (Kannan et al., 2001; Van de Vijver et al., 2003). However, these species, and the same NMMTB archived animals analyzed in the current study, have been used to report baseline levels and temporal trends of other persistent organic pollutants (Peck et al., 2008; Struntz et al., 2004; Tuerk et al., 2005a; Tuerk et al., 2005b). In the one study that analyzed both species, higher levels of hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), dieldrin and toxaphenes were reported in the WSDs than RTDs, and the opposite was observed for mirex (Tuerk et al., 2005a). The authors concluded that the species differences were likely due to spatial differences in chemical use and fate, because the species feed in the pelagic realm at similar trophic levels. Higher levels of polychlorinated biphenyls (PCBs), organochlorine pesticides, and polybrominated diphenyl ethers (PBDEs) were found in immature and adult male WSDs compared to adult females, because of maternal offloading (Tuerk et al., 2005a; Tuerk et al., 2005b). A similar, yet non-significant, finding was seen for hexabromocyclododecanes (HBCDs) (Peck et al., 2008). No significant temporal trend was observed in WSD samples from 1993 to 2004 for HBCDs (Peck et al., 2008) or PBDEs from 1993 to 2000 (Tuerk et al., 2005b). Unexpectedly, POP concentrations did not increase with length or age of male WSDs in this sample set (Tuerk et al., 2005b).

The main two objectives of this study were to 1) estimate the background contamination of PFAAs added to NMMTB samples from materials used during sampling, processing, and long-term archival of tissues; and 2) use the NMMTB samples for exploratory research by investigating PFAA concentrations in 48 WSD and 12 RTD liver samples. Additionally, this study compared PFAA concentrations in paired blubber samples to determine if this lipid rich tissue could be used as a blank tissue for PFAA concentrations in each animal. This hypothesis was justified by known distribution of PFAAs into marine mammal serum and liver rather than lipid-rich blubber (Ahrens et al., 2009b), and because the blubber was processed using the standardized protocols and with the same materials as the liver. The influence of life history traits (species, sex, maturity, animal length) on PFAA concentrations and temporal trends were explored in this dataset which can be expanded in future studies.

2. Material and methods

2.1. Samples

Materials that come into direct contact with marine mammal tissue sampling, processing, and storage at the NMMTB were analyzed for extractable PFAAs at 3M. These included vinyl gloves (glove type #1 used since inception of NMMTB in 1998 to approximately 2005; glove type #2 used from approximately 2003 to 2008) and *co*-polymer gloves (glove type #3 used from approximately 2008 to present) that are worn during handling tissues in the field; Millipore water (18.2 MΩ resistivity) in a PTFE squirt bottle that is used to rinse tissues after collection; additive-free polyethylene film gloves that are worn during cryohomogenization of samples; and fluorinated ethylene propylene (FEP) bags and PTFE jars that are used to store tissues before and after cryohomogenization. Additionally, six aliquots of pooled human serum (20 mL each, Golden West Biologicals Inc.) were provided by 3M to NIST. Three aliquots were cryohomogenized in PTFE disc mills at NIST using identical procedures used for NMMTB tissues (Zeisler

Table 3
Range and median mass fractions (ng/g wet mass) of PFAAs in Atlantic white-sided dolphins and rough-toothed dolphins liver samples.

		White-sided dolphin				Rough-toothed dolphin	
		Female		Male		Male	
		Juvenile ($n = 10$)	Adult (n = 4)	Juvenile (n = 10)	Adult ($n = 23$)	Juvenile (n = 2)	Adult (n = 10)
PFBA	Range (ng/g)	NM	NM	NM	NM	0.759–12.7	<0.485–9.44
	Median (ng/g)					NA	1.84
	Detection frequency					100%	80%
PFOA	Range (ng/g)	<2.57–15.5	<2.83–3.75	<2.53–3.31	<2.50–3.90	1.38–2.47	0.570–2.65
	Median (ng/g)	NA	NA	NA	NA	NA	1.3
	Detection frequency	10%	50%	10%	17%	100%	100%
PFNA*	Range (ng/g)	<2.07–6.56	<2.03–2.35	2.22–8.38	<1.91–9.34	6.09–22.3	4.21–21.0
	Median (ng/g)	2.88	NA	3.23	2.61	NA	6.58
	Detection frequency	80%	50%	100%	70%	100%	100%
PFDA	Range (ng/g)	NM	NM	NM	NM	7.40–20.3	4.14–20.9
	Median (ng/g)					NA	14.9
	Detection frequency					100%	100%
PFUnA	Range (ng/g)	NM	NM	NM	NM	31.7–67.0	14.6–79.1
	Median (ng/g)					NA	57.9
	Detection frequency					100%	100%
PFDoA	Range (ng/g)	NM	NM	NM	NM	5.64–10.1	2.5–11.0
	Median (ng/g)					NA	9.69
	Detection frequency					100%	100%
PFHxS	Range (ng/g)	NM	NM	NM	NM	1.81–16.0	0.444–5.05
	Median (ng/g)					NA	1.56
	Detection frequency					100%	100%
PFOS*	Range (ng/g)	85.3–338	<48.8–59.4	63.5–645	59.0–344	225–1260	51.5–932
	Median (ng/g)	225	53.3	187	115	NA	520
	Detection frequency	100%	75%	100%	100%	100%	100%
PFOSA	Range (ng/g)	NM	NM	NM	NM	41.4–161	25.1–155
	Median (ng/g)					NA	97.0
	Detection frequency					100%	100%

NM not measured; NA not available; * indicates a significant species difference in adult male dolphins, $p < 0.05$.

et al., 1983), and stored in polypropylene bottles. The other three aliquots were stored alongside these samples as blanks. All serum samples were shipped back to 3M for PFAA analysis.

Forty-eight WSD liver samples and three paired blubber samples plus 12 RTD liver samples were retrieved from the NMMTB, relabelled using a randomized blind labeling system, and shipped to 3M for analysis of PFAA mass fractions (concentrations). WSDs were a mixture of males and females and juveniles and adults, which, with the exception of one that stranded in Maine, all stranded in Massachusetts between 1993 and 2005. The dolphins ranged in total length between 150 cm and 271 cm. The RTDs were only males that stranded in Florida between 1997 and 2005 and ranged in length from 207 cm to 254 cm.

2.2. Extraction

Gloves and PTFE bags were cut into known surface area or mass portions and extracted with either 100 mL of water by rotating for 30 min followed by a 12 h static hold or 40 mL of water by shaking for 30 min. PTFE jars were extracted with 5 mL of water. Serum (50 μL) was extracted with 450 μL of acetonitrile using a 96-well protein precipitation plate and a robotic preparation station. All of these samples were spiked with mass-labelled PFAAs before or during extraction.

Liver and blubber samples (1 g or 0.25 g) were extracted using acetonitrile (10 mL or 2.5 mL), spiked with mass-labelled PFAAs, and cleaned up using centrifugation and a solid-phase extraction (SPE) column eluted with 2 mL of methanol. RTD liver extracts did not undergo SPE, because this clean-up step was deemed unnecessary based on results from matrix spikes.

2.3. Quantification

Quantification of PFAAs was conducted using liquid chromatography (API 4000) triple quadrupole mass spectrometry (Applied Biosystems/Sciex) in negative electrospray ionization mode. Replicates, blanks, and matrix spikes were used as quality assurance and quality control. PFOS, PFOA, and PFNA were quantified in the NMMTB sampling and homogenization materials and WSD tissues; whereas a larger suite of compounds were quantified in the RTDs. The background estimates measured from sampling and homogenization supplies were not subtracted from the dolphin data.

2.4. Statistical analysis

The open-source program R (http://cran.r-project/org [R Development Core Team, 2005]) was used for all statistical analyses and visualizations (primarily packages “dplyr”, “ggplot2”, and “NADA”). Concentration data were grouped by species, age class, and sex. Median, range, and detection frequency were used as descriptive statistics for all data sets due to small, unbalanced sample sizes with variable detection frequencies. Estimators for median recommended by Helsel (2005) as implemented in NADA were used for data sets with <100% detection frequency; in brief, data sets with >50% detection used the Kaplan-Meier estimation, data sets with 20% to 50% detection used robust regression on order statistics, and only the range is reported for data sets with <20% detection. Significance levels for all hypothesis tests were set at $\alpha = 0.05$. The ability to conduct statistical analysis was somewhat limited as a consequence of opportunistic sampling from rarely encountered species; limitations are described below.

Comparisons between species was limited to PFNA, PFOA, and PFOS in adult males, and comparisons between size class and sex were limited to WSDs. Data were visualized using standard Tukey-style box plots where boxes represent the interquartile range (IQR), the median (thick black line inside the box), and whiskers extend to the lesser of 1.5*IQR and the maximum/minimum observation. For data sets with 100% detection, *t*-tests (two groups) or ANOVAs (three or more groups) were used for hypothesis testing when groups fit assumptions of normality and homoscedasticity. Significant ANOVAs were followed by pairwise Tukey's HSD. Wilcoxon rank-sum tests were used when data sets required nonparametric tests. Significant results when comparing three or more groups were followed by pairwise Wilcoxon rank-sum tests. When detection in at least one of the groups was <100%, empirical cumulative density functions (ECDFs) were compared using the Peto-Peto modification of the Gehan-Wilcoxon test (significant results were followed by pairwise ECDF comparison).

Regression against animal total length in cm was limited to WSDs. This analysis was not performed on RTD because the small sample size provided only two juveniles and limited the range of animal lengths. Concentrations were visualized using a locally weighted scatterplot smoother (LOESS) with 95% confidence interval. Hypothesis testing of data sets with 100% detection used windowed generalized linear models (GLM) based on age class (cuts based on length as described in the introduction). Hypothesis testing of data sets with <100% detection used Kendall's τ regression, visualized with the Akritas-Thiel-Sen and Turnbull estimates of slope and intercept, respectively.

Testing approaches for temporal trends were also necessarily different for each species as only samples from adult male WSDs provided adequate temporal coverage for regression analysis. To provide a continuous numerical value, stranding dates were converted to the integer number of days since 30 Dec 1899 as per R documentation. Hypothesis testing was largely as described above for length. Due to the change in PFOS production circa 2001 and 100% detection, the same windowed GLM approach was used with the window break set at 13 April 2001; other PFAAs were not windowed. Trends were visualized similarly to length, with a LOESS (and 95% confidence interval) curve. Because

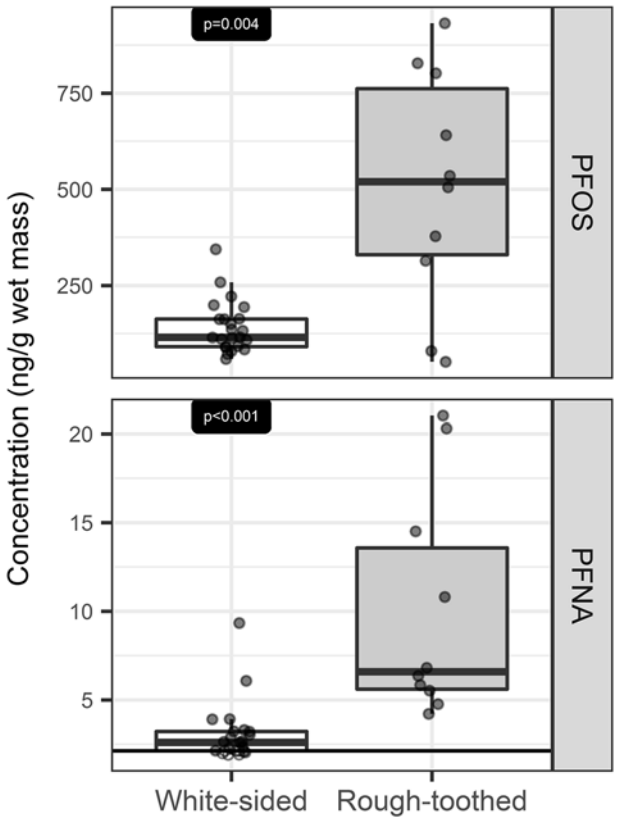

Fig. 1. Species differences of PFOS and PFNA mass fractions (ng/g wet mass) in liver of adult male Atlantic white-sided dolphins (n = 23) and rough-toothed dolphins (n = 10). Open circles represent samples below the limit of detection, solid horizontal lines across the entire plot represent the maximum limit of detection. P-values below 0.05 (shown in black boxes) were considered significant from Peto-Peto modification of Gehan-Wilcoxon tests comparing the two species. Additional plot parameters are described in Material and Methods: Statistical analysis.

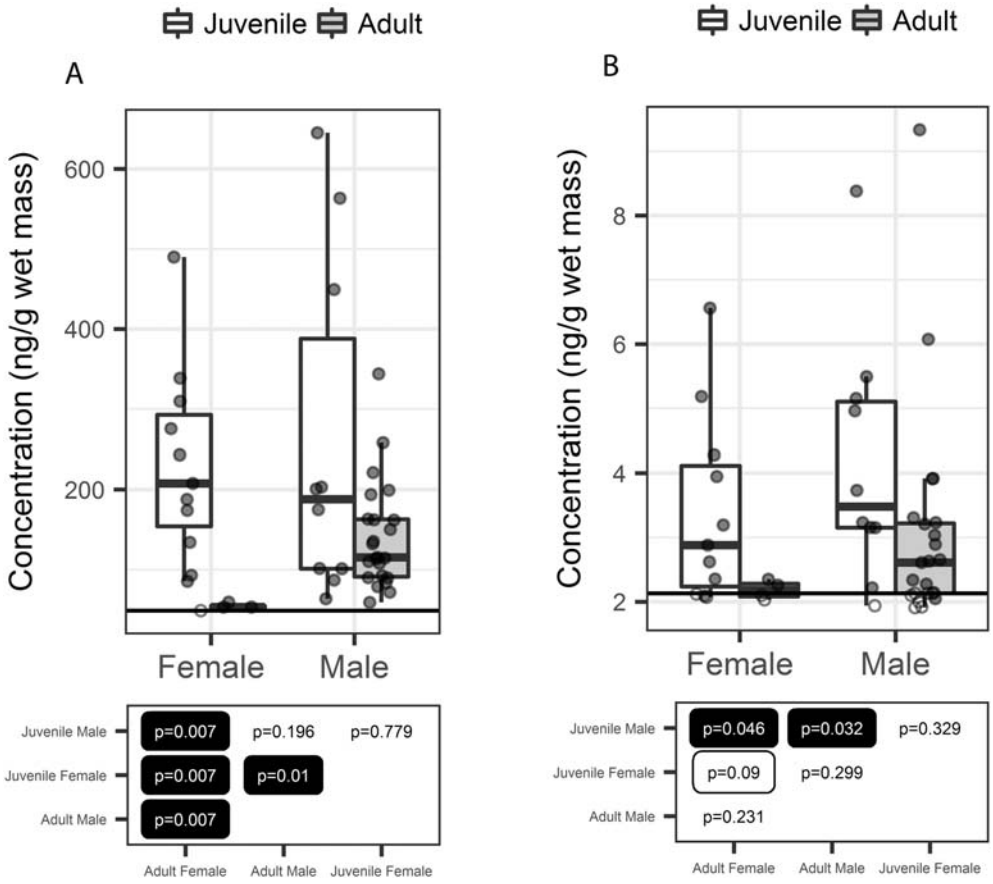

Fig. 2. Sex and life stage differences of (a) PFOS and (b) PFNA in Atlantic white-sided dolphin liver samples. Open circles represent samples below the limit of detection, solid horizontal lines across the entire plot represent the maximum limit of detection. *P*-values below 0.05 (shown in black boxes) were considered significant from pairwise comparisons of the different groups of sex and age classes. Statistical tests chosen and additional plot parameters are described in Materials and Methods: Statistical analysis.

stranding events for RTDs are rare, only samples from adult males were adequate to conduct a temporal analysis, and they grouped naturally into three year-classes (1997, 2001, and 2004/2005). Rather than attempt a true regression analysis, these were tested by group-wise comparison of year-classes. Hypothesis testing of data sets with 100% detection used ANOVA with relaxed assumptions and, when H_0 was

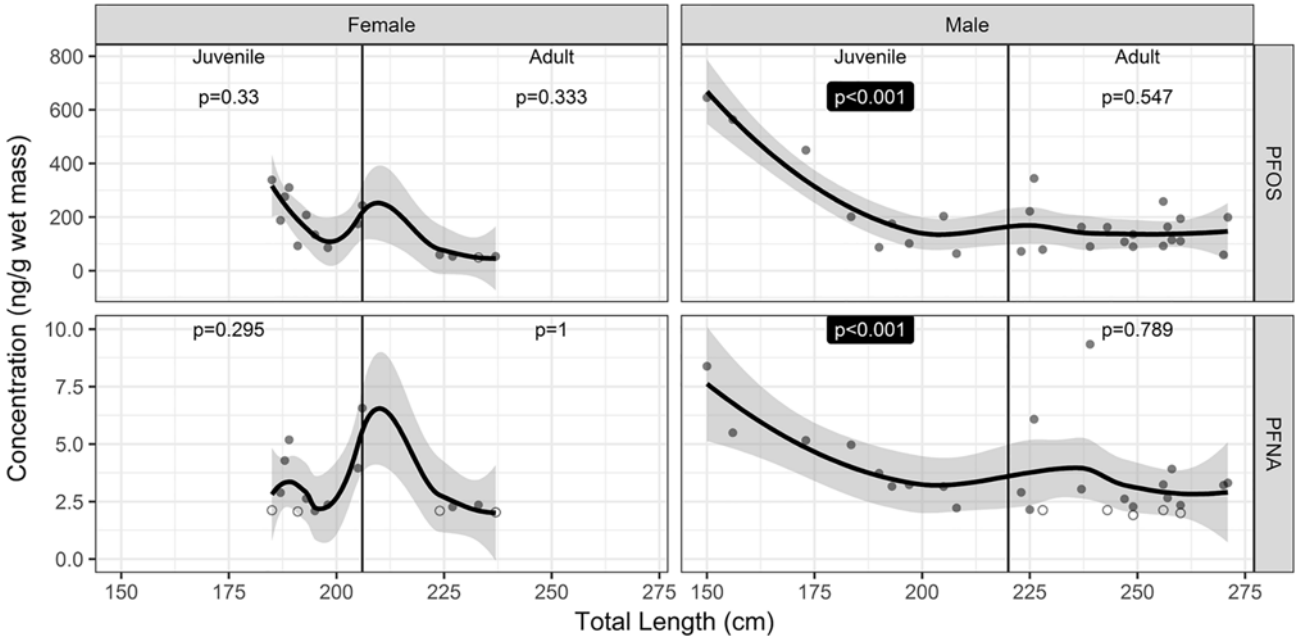

Fig. 3. Relationship of PFOS and PFNA mass fractions (ng/g wet mass) in liver based on animal length in stranded Atlantic white-sided dolphins. Open circles represent samples below the limit of detection. Additional plot parameters are described in Materials and Methods: Statistical analysis.

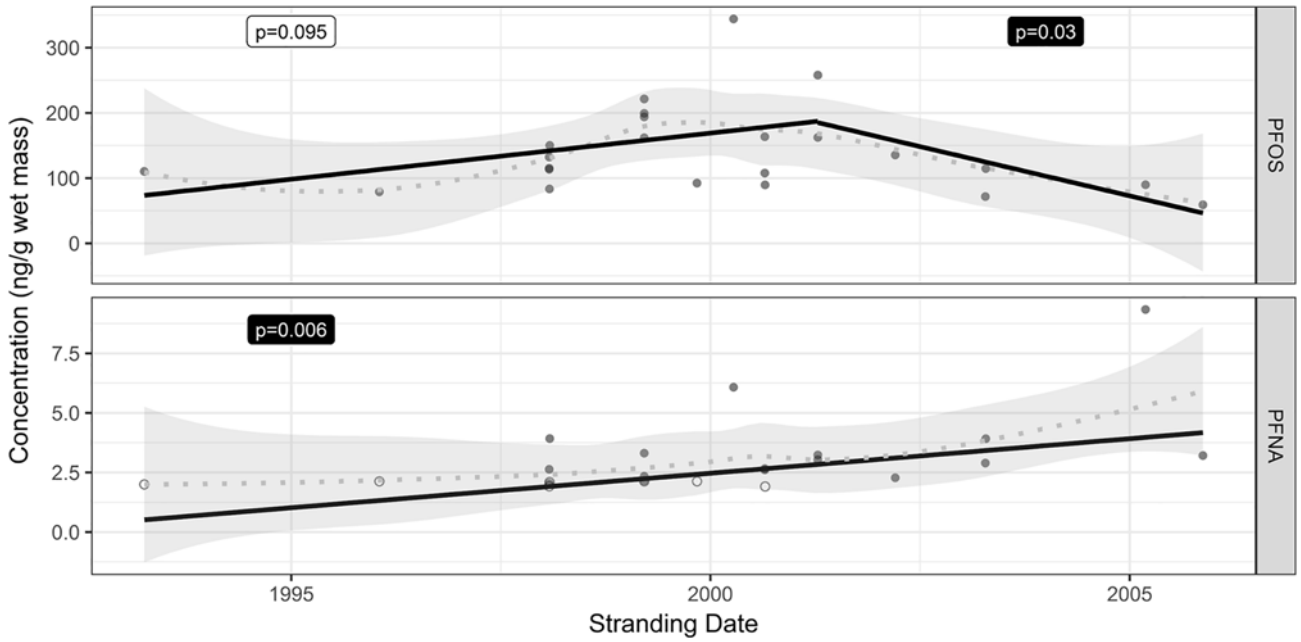

Fig. 4. Time trends of PFOS and PFNA mass fractions (ng/g wet mass) in liver samples from adult male Atlantic white-sided dolphins. Open circles represent samples below the limit of detection. Additional plot parameters are described in Materials and Methods: Statistical analysis.

rejected, testing was followed by post-hoc pairwise Tukey's HSD tests. Hypothesis testing for data sets with <100% detection used the ECDF comparison described above and, when H_0 was rejected, testing was followed by post-hoc pairwise ECDF comparisons between year-classes. Due to the limitations described above, the combination of length and stranding date as predictor variables was not evaluated.

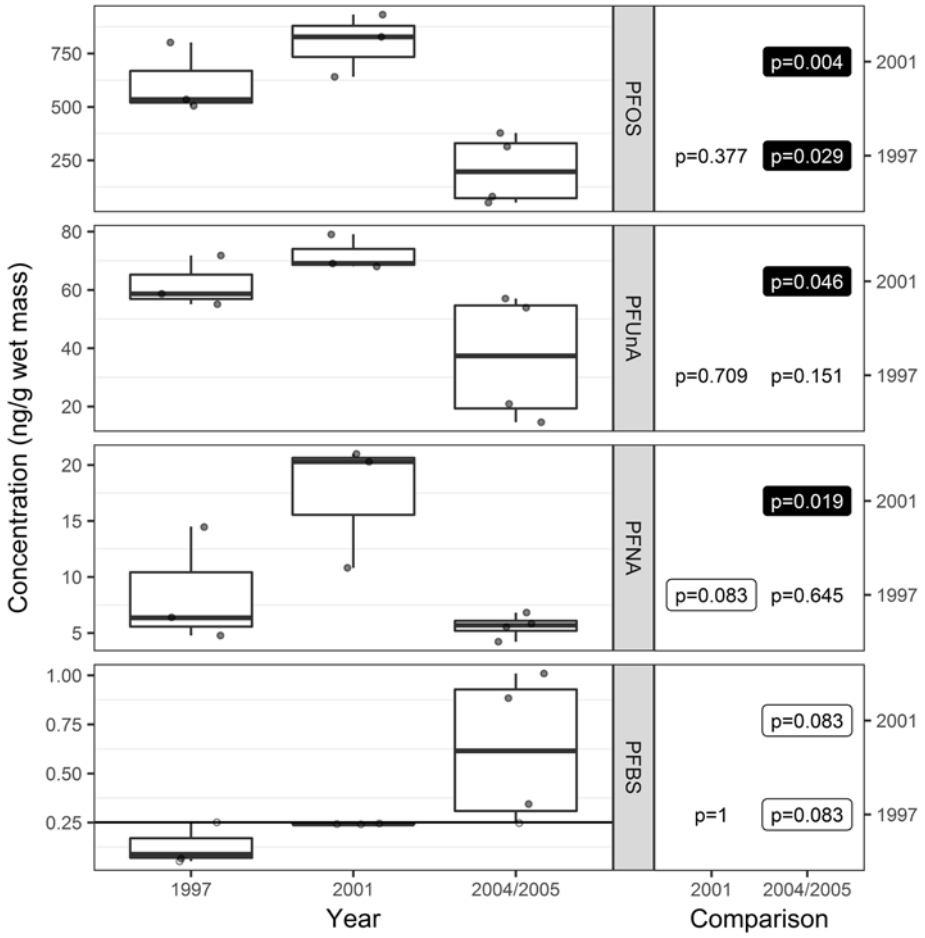

Fig. 5. Mass fractions (ng/g wet mass) of four PFAAs differed by year in liver samples from stranded adult male rough-toothed dolphins. Open circles represent samples below the limit of detection, solid horizontal lines across the entire plot represent the maximum limit of detection. Additional plot parameters are described in Materials and Methods: Statistical analysis.

3. Results and discussion

3.1. Background contamination of PFAAs from material processing

Since PTFE is the main material used for NMMTB protocols, it was important to examine the potential transfer of PFAAs from these materials into samples. PFAAs were extractable from most of the sampling, processing, and storage materials (Table 1). The three types of sampling gloves used at different times through the collection of NMMTB samples had differing PFAA concentrations with glove type 2 used between 2003 and 2008 containing the highest concentrations of PFAAs. Small amounts of PFAAs were introduced into the cryohomogenized serum samples. Using these concentrations and estimates of surface area, volume, and time that these materials touch samples, approximate contamination of samples by materials during sampling, processing, and cryohomogenization techniques were calculated (Table 1). Assumptions were made so that values are likely overestimated. <1 ng/g of PFOA is expected to leach from materials into samples, with much lower transfer of PFNA and PFOS. Stringent, consistent protocols used by the NMMTB since inception were designed to result in minimal and consistent contamination of samples by compounds; however, the required switch between sampling gloves type 1 and 2 around 2003 may have increased PFOS and PFOA contamination in samples collected between 2003 and 2008. Fortunately, this contamination level dropped again around 2008 when glove type 2 was discontinued and glove type 3 was substituted. Background contamination caused by the specimen banking protocols appears to be negligible for PFOS and PFNA for the dolphin liver samples as the minimum concentrations (see below) are at least an order of magnitude higher than transfer contamination. On the other hand, PFOA background contamination (up to 0.968 ng/g) could represent a significant portion of the PFOA measured in the dolphin liver samples. These data indicate that it is indeed feasible to examine PFAAs in NMMTB archived tissues, but emphasize the importance of understanding potential background contamination from the sampling, homogenization and storage procedures.

For this reason, it was important to consider this background contamination for data in two previously published studies from the NMMTB on northern fur seals (*Callorhinus ursinus*) and beluga whales (*Delphinapterus leucas*) (Reiner et al., 2016; Reiner et al., 2011). Those studies did not report PFOA concentrations because they were below either the detection limits or these background levels. The minimum detected concentrations of PFNA were 0.17 ng/g in beluga whales and 0.555 ng/g in northern fur seals, which are two- to six-times greater than the estimated background from supplies. For PFOS, the marine mammals were four- to eight-times greater than the background levels. Thus, the background contamination may have had a minor effect on the lowest reported concentrations, but little to no effect on the means.

The three WSD blubber samples analyzed contained detectable levels of PFOS and PFNA, but not PFOA (Table 2). Initially, we hypothesized that low blubber PFAA concentrations might provide an estimate of background contamination from banking materials, which could be subtracted from the paired liver sample. However, this was not the case. PFOS concentrations in the blubber (minimum 14 ng/g) are orders of magnitude higher than estimated contamination from materials (up to 0.221 ng/g). The comparison of PFNA is similar. This suggests that PFAA concentrations measured in the blubber are from bioaccumulation by the animal and distribution into this lipid-rich tissue through either protein components or blood. Blubber concentrations of PFOS and PFNA were only 10.5% and 15.0% of the liver concentrations, respectively. Blubber concentrations of PFAAs have been measured in other marine mammals, including harbor seals (*Phoca vitulina*) from the German Bight (PFOS range 0 ng/g to 23 ng/g) and Wadden Sea (18.9 ng/g to 296.9 ng/g) (Ahrens et al., 2009b; Van de Vijver et al., 2005) and ringed seals (*Phoca hispida*) from the Canadian Western Arctic (0.4 to 0.9 ng/g) (Powley et al., 2008). Similar to these studies, PFAAs were detected in low amounts in the dolphin blubber samples compared to their paired liver samples.

3.2. PFAA exploratory research using banked dolphin samples

At least one PFAA was detected in each of the dolphin samples (Table 3, Supporting Information Tables S1 and S2). PFOS was detected in the highest frequency (> 98% of the samples) and at the highest concentrations (up to 1260 ng/g). PFNA was also detected frequently (>80% of the samples), but was much lower in concentration (up to 22.3 ng/g) compared to PFOS measured in the same samples. PFOA was detected in few of the WSDs (17% of the samples) and at concentrations too low and close to the background contamination from supplies (0.968 ng/g) in both species, negating further consideration or interpretation of the PFOA data in this study. RTD samples (but not WSDs) were analyzed for additional PFAAs with PFOS having the highest median concentration (520 ng/g in adults) of all PFAAs, followed by PFOSA (97.0 ng/g). PFUnA (median = 57.9 ng/g in adults) was the predominant perfluorocarboxylic acid (PFCA).

3.2.1. Species differences suggest spatial differences in PFAA contamination

Given the dataset restrictions, the only species comparison that can be discussed is between adult males for PFOS and PFNA. The more northerly WSDs had roughly half the concentrations of PFOS and PFNA compared to the more tropical RTDs (Table 3, Fig. 1). Since these species are similar in their offshore pelagic life history and trophic level, the differences are more likely caused by spatial differences in contamination and fate of PFAAs. Most studies addressing spatial trends in PFAA contamination focus on the Arctic and few are available in the tropical or temperate regions of the North Atlantic. Two studies on nearshore foraging marine species, like loggerhead sea turtles (*Caretta caretta*) (O'Connell et al., 2010) and bottlenose dolphins (*Tursiops truncatus*) (Houde et al., 2005) show the opposite spatial patterns with higher levels in northern sites, like the Chesapeake Bay or Charleston, South Carolina, compared to southern sites, like Florida Bay or Sarasota Bay in the Gulf of Mexico. It is possible that these coastal species reflect more local, land-based sources of PFAAs; whereas the offshore WSDs and RTDs of the present study reflect PFAA concentrations in deep, pelagic waters. In fact, open-ocean surface seawater sampling supports this idea with North Atlantic Ocean water at locations within the WSD distribution having lower PFOS concentrations than equatorial Atlantic Ocean water (Yamashita et al., 2005). The opposite latitudinal trend has been seen in surface seawater samples but the sampling design was confounded by distance from shore (Benskin et al., 2012). In their study, northerly samples, nearshore Rhode Island and on the continental shelf, had higher concentrations than offshore southerly samples, suggesting that distance from shore was a stronger influence than latitude within their sample set. A previous comparison between these two species for most other POPs differs from the PFAAs (Tuerk et al., 2005a). For example, both species had similar total PCB, total DDT, total chlordane, and total PBDE concentrations, but the northern WSD had higher concentrations of total HCHs, HCB, dieldrin, and total toxaphene than RTDs. The only result from Tuerk et al. (2005a) that is similar to the PFAAs is that WSDs had lower concentrations of mirex than RTDs, and this was thought to be due to greater usage of mirex in southern latitudes.

3.2.2. Atlantic white-sided dolphins: sex and life stage differences

Significant sex and life stage differences were observed for PFOS and PFNA concentrations in WSD liver samples (Fig. 2). Adult males had higher concentrations than adult females of PFOS, but this trend was not significant for PFNA. Maternal offloading to fetal tissues or through lactation could partly explain the PFOS finding (Fair et al., 2012; Hart et al., 2008; Reiner et al., 2011), but sex differences in elimination rates are also possible. For both compounds and within each sex, juveniles tended to have greater levels than adults with significant

differences for PFOS in females and PFNA in males. This change through age was further assessed by regressions between PFAA concentrations and animal length (Fig. 3). As observed in several other studies (Ahrens et al., 2009a; Dassuncao et al., 2017; Fair et al., 2012; Hart et al., 2008; Houde et al., 2006; Reiner et al., 2011; Tao et al., 2006) juveniles can have higher concentrations than adults and appear to exhibit growth dilution for both PFNA and PFOS until levels stabilize in adulthood (Fig. 3).

3.2.3. Temporal trends

Temporal trends were assessed in only adult male WSDs to minimize confounding influences from sex and life stage differences (Fig. 4); however, data are plotted across years for other ages and sexes in Supporting Information (Fig. S1). PFOS did not significantly increase or decrease before 2001, but significantly declined after 2001. The rate of annual decline post-2001 was 22.6%, which was calculated using a linear regression of the natural log of the PFOS concentrations to stranding year. A similar decreasing rate of 20% per year was observed in loggerhead sea turtles along the U.S. eastern coast (O'Connell et al., 2010). Declines in PFOS are expected in response to the reduction in manufacturing of PFOS-related chemicals in the U.S. PFNA increased significantly throughout the sample collection years at 15.8% annually, which is similar to increasing trends seen in Alaskan marine mammals for total PFAAs in northern fur seals (1987–2007; 9% increase per year driven mainly by PFCAs not PFOS) (Reiner et al., 2016) and for PFNA in beluga whales (1989–2006) (Reiner et al., 2011), but opposite to the decreasing trend seen in loggerhead sea turtles (O'Connell et al., 2010). These comparisons within the literature suggest that time trends can be species and location dependent.

The RTDs represented only three discrete stranding events (1997, 2001, and 2004–2005) prohibiting the assessment of a continuous temporal trend. However, we did observe significant differences among these events for PFOS, PFNA, and PFUnA concentrations (Fig. 5). Dolphins that stranded in 2001 had significantly higher concentrations of these three compounds than those that stranded in 2004/2005. Perfluorobutane sulfonate (PFBS) concentrations showed an increasing trend across the years, but these were not significant. These temporal trends are in the expected direction based on manufacturing rates but are limited by small sample sizes ($n = 3$ or 4 animals per event) and confounded by location differences. RTDs sampled in 1997 and 2001 stranded along the panhandle of Florida in the Gulf of Mexico, whereas RTDs from 2004/2005 stranded on Hutchinson Island and Marathon Key on the Atlantic coast of Florida. As additional animals from this species are archived by the NMMTB, a better understanding of changes through time can be ascertained. Concentration data for individual compounds and animals are provided in Supporting Information (Table S2) so that future studies can expand this dataset.

4. Conclusion

This study demonstrates the importance of understanding background contamination of emerging compounds of concern that can leach from sample collection, processing, and storage supplies. Only after understanding the potential background contamination can one leverage samples from a specimen bank to do exploratory research. We have shown that samples from these two dolphin species archived in the National Marine Mammal Tissue Bank are feasible for assessing trends in concentrations of PFAAs, except for PFOA. The exploratory research on life history, spatial, and temporal trends in these two species can aid future study designs. Retrospective studies should consider selecting only one sex and life stage of a species for analysis to avoid the documented influence of those factors on the accumulation of PFAAs. Future studies might also focus on a species that spans both spatial locations, perhaps the bottlenose dolphin, to better understand the spatial gradient of PFAA contamination along the U.S. east coast. In addition, future studies could expand the sample size of these species as additional samples are archived in the tissue bank to obtain an even longer temporal trend for PFAAs.

Disclaimer

Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.11.299.

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