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Size and age–concentration relationships for perfluoroalkyl substances in stingray livers from eastern Australia

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HIGHLIGHTS

- Hepatic concentrations of nine PFASs have been measured in 49 stingrays from East Australia.
- Negative correlation between the concentration of PFASs in the livers of 32 blue-spotted stingrays and the body size /age is found.
- Dependence on body size (and age) may be related to different uptake kinetics of the chemicals after an increased level of the contaminants in the environment.

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ABSTRACT

While the literature has reported a widespread occurrence of perfluoroalkyl substances (PFASs) in marine biota, very limited studies have been dedicated to the southern hemisphere. Hepatic concentrations of nine PFAAs were analysed in 49 stranded stingrays from eastern Australia using liquid chromatograph coupled with tandem mass spectrometry and relationships with biological parameters (i.e. body size, age and sex) were investigated. Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) were the predominant compounds quantified, with hepatic concentrations varying from 2 to 117 and from 0.2 to 19 $ng \cdot g^{-1}$ w.w., respectively. A negative correlation between the concentration of PFASs in the livers of 32 blue-spotted stingrays and the body size/age was found. This relationship was independent of the animal's sex. We postulate that the dependence on body size is related to differing uptake kinetics of the chemicals, after the sting rays were exposed to an increased level of the contaminants in their environment. Such a pollution event could be related to a severe flood event that occurred at this location a few months before the samples' collection. Our results indicate that the influence of the body size/age should be taken into account when estimating bioaccumulation parameters from environmental measurements or exposure levels of biota to PFASs.

1. Introduction

Perfluoroalkyl substances (PFASs) are a class of synthetic chemicals that have been detected globally in a wide range of environmental (e.g. air, water, sediment) and biological matrices (e.g. human, wildlife and fish) (Lindstrom et al., 2011). For 60 years, these chemicals have been manufactured and used in numerous consumer products and exploited for their exceptional surface-active properties, water and oil repellence, and chemical stability. Due to the high energy of the covalent C – F bond, PFASs are resistant to degradation processes, which results in the environmental persistence and bioaccumulative potential of these chemicals. Water is the most important medium for PFAS transport in the environment, which makes water bodies a major sink for PFASs (Ahrens, 2011; Ahrens et al., 2010; Yamashita et al., 2005, 2008) and results in PFAS exposure to aquatic organisms (Giesy and Kannan, 2001; Sturm and Ahrens, 2010).

PFASs have been detected in a wide range of aquatic species, particularly in liver tissue where they primarily tend to bioaccumulate (Ahrens et al., 2009; Ishibashi et al., 2008; Peng et al., 2010; Quinete et al., 2009). The most frequently detected compounds are perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs) of varying chain lengths. These can be directly emitted into the environment but also be the biodegradation products of several volatile polyfluorinated compounds, such as polyfluoroalkylated sulfonamides, sulfonamidoalcohols, and fluorotelomer alcohols (Buck et al., 2011).

Trophic biomagnification of perfluorooctane sulphonate (PFOS) and long-chain PFCAs has been observed in polar, temperate and subtropical food webs (Houde et al., 2006; Kannan et al., 2005; Kelly et al., 2009; Loi et al., 2011). With PFOS having been identified as the predominant chemical found in biota and in any tissue analysed (Houde et al., 2011). The highest PFOS contamination has been found in fish livers from more industrialised regions such as North America (up to 315 $ng \cdot g^{-1}$ wet weight (w.w.) in New York inland lakes) (Sinclair et al., 2006), the Sea of Japan (up to 3250 $ng \cdot g^{-1}$ w.w.) (Taniyasu et al., 2003) and Europe (up to 7760 $ng \cdot g^{-1}$ w.w. in the North Sea (Hoff et al., 2003)), while the lowest concentrations of PFOS were

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detected in fish livers from pristine regions like in polar area or Tibetan plateau (up to 5 $ng \cdot g^{-1}$ w.w. (Haukås et al., 2007; Shi et al., 2010)). Concentration of PFASs in offshore ocean water has been shown to be lower in the Southern Hemisphere than in the Northern Hemisphere (Yamashita et al., 2008) but little data is currently available on the presence and abundance of these compounds in fish tissues in the Southern Hemisphere. Indeed, to our knowledge, only a limited studies on marine biota have been undertaken, in Brazil (Quinete et al., 2009) and in Australia (level up to 107 $ng \cdot g^{-1}$ w.w. in sea mullet liver sample) (Thompson et al., 2011).

Investigations on toxicology have been carried out and PFASs, particularly PFOS, have been shown to induce developmental delays, neonatal mortality, and carcinogenic effect in animal laboratory studies (Lau et al., 2007). The chemical concentration in biota tissues is an important component to assess relationships between level of exposure and potential health impacts. Assessing exposure levels of a contaminant in biological samples can be strongly dependent on biological parameters such as size, sex, age, and metabolic activities (Houde et al., 2011). Other organohalogen compounds such as polychlorinated biphenyls have been described, with links between concentration and the size or age of fish (Gewurtz et al., 2011). Studies have reported sex-specific differences in concentrations of these persistent chemicals in marine mammals, with females generally contain relatively lower concentrations due to a transfer to the offspring, while the concentrations in males tend to increase with age (Borrell et al., 1995). However, the influence of such biological variables in accumulated PFAS levels in fish tissues has rarely been investigated. Concentration of PFOS, in female bass livers was observed at a lower level than in males (Sinclair et al., 2006), however, the difference between sex was not significant in skipjack tuna livers from the Sea of Japan ($p \geq 0.05$) (Hart et al., 2008). The highest concentrations of longer chain PFCAs were recorded in the long lived Chinese sturgeon and linked to the bioaccumulation potential of these longer chain compounds, but no significant correlation with age was found for PFOS (Peng et al., 2010). A maternal transfer for PFASs in fish through oviparous elimination was assumed due to higher PFOS concentrations in fish eggs (Kannan et al., 2005; Peng et al., 2010; Sharpe et al., 2010) but this has not been currently investigated for aplacental viviparous species such as rays and sharks. Overall, there is a lack of trend in the relationship between age, size and accumulation of PFASs as well as sex differences reported in the literature.

In this study we measured the concentration of PFCAs (C6–C13) and perfluorinated sulfonic acids PFSAs (C6 and C8), in the liver of stingrays with the aim of assessing whether we can identify and potentially explain differences in concentrations between species as a function of age/size and sex of the individuals.

2. Materials and methods

2.1. Sampling and population

A total of 49 stingrays were found freshly stranded on the west coast of Stradbroke Island (centred on 27°29′25″S; 153°24′18″E within the Moreton Bay) in South East Queensland, Australia, in September 2012. Taxonomic keys were used to identify individuals to the species level (Last and Stevens, 2009). Two different families of stingrays were identified, including Myliobatidae with three white-spotted eagle rays (*Aetobatus narinari*) and the Dasyatidae family including 1 estuary stingray (*Dasyatis fluviorum*), 6 black-spotted whiprays (*Himantura astra*), 4 brown whiprays (*Himantura toshi*), 1 reticulated whipray (*Himantura uarnak*) and 34 blue-spotted rays (*Neotrygon kuhlii*). All six of the species are viviparous, with the developing embryos held within the mother's body nourished at first by yolk and later by histotroph ("uterine milk"), with no placental connection (Last and Stevens, 2009).

Liver tissues were dissected out right after they were stranded, then kept in polyethylene bags and stored at −20 °C until analysis. The sex and disc width (WD), the widest portion of the disc, from wing tip to wing tip (±1 mm), were recorded for each individual. The mean trophic levels of the Dasyatidae and Myliobatidae families are respectively 3.62 (based on 25 species varying between 3.16 and 4.08) and 3.37 (varying between 3.10 and 3.72 for 8 species), with all species known to actively hunt for prey in soft bottom environments and feed primarily on crustaceans, molluscs, and some bony fish (Jacobsen and Bennett, 2011; Last and Stevens, 2009).

2.2. Age estimation

The three-parameter Von Bertalanffy growth function (VBGF) (Beverton and Holt, 1957; von Bertalanffy, 1938) was used to estimate the age of stingrays based on their disc width (WD), with distinct parameter values for females and males. The age ($\boldsymbol{t}$) is related to the disc width ($\boldsymbol{WD\ t}$) by Eq. (1) with the following parameters: $\boldsymbol{WD\ t}$ (mean WD at age t); $\boldsymbol{WD\ \infty}$ (theoretical asymptotic WD); **Kg** (growth coefficient) and $\boldsymbol{t_0}$ (theoretical age at zero WD):

$$\boldsymbol{t} = \boldsymbol{t}_0 - \left(\frac{1}{\boldsymbol{Kg}}\right) \times \ln\left(1 - \frac{\boldsymbol{WD\ t}}{\boldsymbol{WD\ \infty}}\right). \quad (1)$$

The following parameters were determined for female blue-spotted rays ($\boldsymbol{WD\ \infty}$ = 465.81 mm, **K** = 0.13 $year^{-1}$, $\boldsymbol{t_0}$ = −3.51 year) and male blue-spotted rays ($\boldsymbol{WD\ \infty}$ = 385.19 mm, **K** = 0.20 $year^{-1}$, $\boldsymbol{t_0}$ = −3.07 year) (Pierce and Bennett, 2009) and used in this present study. The three-parameter Von Bertalanffy growth function provides a good statistical fit to size at age data in both sexes, with a coefficient of determination R^2 of 0.92 for females and R^2 = 0.88 for males (Pierce and Bennett, 2009). Black-spotted whiprays and brown whiprays are closely related to each other and present similar morphology, so the parameters found in Jacobsen and Bennett (2011) have also been used to estimate the age for both species. The age of Estuary stingray has been determined using the parameters of Pierce and Bennett (2010). No published age estimate calculations could be found for the reticulated whipray nor the white-spotted eagle ray.

2.3. Chemicals and reagents

The PFACs investigated in this work are perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA) and perfluorotridecanoate (PFTrDA). The analysed PFASs are perfluorohexanesulfonate (PFHxS) and perfluorooctanesulfonate (PFOS). The native PFAC and PFSA solution mixture (MPFAC–MXB) were purchased from Wellington Laboratories, Guelph, Ontario, Canada. The ^{13}C-labelled PFAC and PFAS solution mixture (MPFAC–MXA) was obtained by Wellington Laboratories, Guelph, Ontario, Canada and contained: perfluoro[1,2,3,4-$^{13}C_4$]butanoic acid, perfluoro[1,2-$^{13}C_2$]hexanoic acid, perfluoro[1,2,3,4-$^{13}C_4$]octanoic acid, perfluoro[1,2,3,4,5-$^{13}C_5$]nonanoic acid, perfluoro[1,2-$^{13}C_2$]decanoic acid, perfluoro[1,2-$^{13}C_2$]undecanoic acid, perfluoro[1,2-$^{13}C_2$]dodecanoic acid, sodium perfluoro-1-hexane[$^{18}O_2$]sulfonate, and sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate. A ^{13}C-labelled instrument performance internal standard perfluoro[$^{13}C_8$]octanoic acid ($^{13}C_8$-PFOA) and perfluoro[$^{13}C_8$]octanesulfonate ($^{13}C_8$-PFOS) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Methanol (MeOH) and acetonitrile (ACN) in LC-grade were acquired from LiChrosolv. Hydrochloric acid (HCl, 37%) was obtained from Riedel-de Haën (Seelze, Germany).