



Per- and polyfluoroalkyl substances (PFAS), trace elements and life history parameters of mass-stranded common dolphins (*Delphinus delphis*) in New Zealand

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ABSTRACT

Profiles of 33 PFAS analytes and 12 essential and non-essential trace elements were measured in livers of stranded common dolphins (*Delphinus delphis*) from New Zealand. PFAS concentrations reported were largely comparable to those measured in other marine mammal species globally and composed mostly of long-chain compounds including perfluorooctanesulfonic acid (PFOS), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTTrDA) and perfluorooctanesulfonamide (FOSA). PFAS profiles did not vary significantly by location, body condition, or life history. Notably, significant positive correlations were observed within respective PFAS and trace elements. However, only negative correlations were evident between these two contaminant types, suggesting different exposure and metabolic pathways. Age-associated concentrations were found for PFTTrDA and four trace elements, i.e. silver, mercury, cadmium, selenium, indicating differences in the bioaccumulation biomagnification mechanisms. Overall, our results contribute to global understanding of accumulation of PFAS by offering first insights of PFAS exposure in cetaceans living within South Pacific Australasian waters.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) have a global occurrence (Glüge et al., 2020; Miner et al., 2021; Routti et al., 2016) and represent some of the most notable emerging contaminants of concern (Nakayama et al., 2019). Manufactured since the 1940's, these substances have high stability, and are repellent to either water or oil (Escoruela et al., 2018). Such properties make them ideal for industrial, commercial, and even medical use (Galatius et al., 2013; Glüge et al., 2020). Surfactants, pesticides, fluorinated polymer feedstock and process aids, fire-fighting

foams, and even food packaging represent examples of their wide use (Renner, 2006). Based on the available records, the Organisation for Economic Co-operation and Development (OECD) compiled a list of more than 4700 PFAS chemicals on the global market (OECD, 2018), some of which pose greater risk to human and wildlife health than others (Brown et al., 2020; Death et al., 2021; Ding et al., 2020; Kim et al., 2020; Lohmann et al., 2020), and further complicated in the context of assessing single chemical versus whole mixture toxicity assessments (Goodrum et al., 2021; Kim et al., 2021). With over 200 known industrial applications to date, many PFAS are emitted either directly into the

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environment during manufacturing processes (Paul et al., 2009) or indirectly via transport and transformation of PFAS-containing products.

Some PFAS substances bioaccumulate and are subject to long range transport (Kannan et al., 2001; Miner et al., 2021; Xie et al., 2015). Perfluoroalkyl acids (PFAAs), a sub-group of PFAS including perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are considered indefinitely persistent. Many other PFAS that can transform in the environment to form PFAAs, are known as precursors to PFAAs. Only a few PFAS are currently restricted by regulation. PFOS and PFOA are the most studied and are now listed under the Stockholm Convention (UNEP, 2009). While PFOS use in New Zealand was phased out as of 2011, some applications of PFOA have remained possible until recently (HSNO, 2017). Furthermore, reduction in manufacturing and usage is not global, with many Asian countries dramatically increasing their production because of phase-out initiatives in North America (Routti et al., 2016). An associated rise in alternative PFAS compounds has additionally been noted, which is of further concern as the environmental and biological implications of such alternatives are even less well understood (Gallen et al., 2018; Möller et al., 2010; Zhang et al., 2020). The impact of these changes on the environment and human health in the Australasian region is also unknown.

PFAS are persistent in the marine environment and can accumulate in biota (Jensen and Leffers, 2008). For example, PFOS distributes to the plasma and kidneys due to its affinity for blood and proteinaceous tissues and is eventually excreted slowly through the liver and urine with a half-life of 8.67 years (Hekster et al., 2003; Houde et al., 2005). While long chain PFAAs are biologically active and associated with health conditions including reproductive toxicity, endocrine disruption, hepatotoxicity, neurotoxicity and immunotoxicity (e.g., Blake and Fenton, 2020; Di Nisio et al., 2019; Foresta et al., 2018; Sunderland et al., 2018), the health implications for most other PFAS remains unclear. However, recent experimental toxicity testing conducted within Australia demonstrate toxicity of PFOS to amphipod survival and reproduction in waters off New South Wales (Simpson et al., 2021). Additional neuro-behavioral development and thyroid disruption has also been identified across various species (e.g., Blake and Fenton, 2020; Kim et al., 2020). As apex predators, marine mammals are reliable environmental sentinels for both ocean and human health (Bossart, 2010), with concentrations of many anthropogenic chemicals being comparatively high in both dolphin and human populations (Backer et al., 2019; Wang et al., 2021).

Kannan et al. (2001) first reported PFOS in tissues of 15 marine mammal species worldwide and it has subsequently been detected internationally across a broad range of marine mammal taxa (e.g., Andvik et al., 2021; Dassuncao et al., 2018; Escoruela et al., 2018; Fujii et al., 2018; Taylor et al., 2021; Wang et al., 2021; Zhang et al., 2021) including those within Arctic and Antarctic waters (e.g., Routti et al., 2019; Sonne et al., 2021). Indeed, considerable historical PFAS research effort has focused on polar regions (Escoruela et al., 2018), though the occurrence and concentrations of PFAS has been increasingly observed in temperate and tropical marine mammal species in the last decade (Moon et al., 2010). For example, studies of Indo-Pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*) revealed significant increasing trends of PFAS concentrations (Lam et al., 2016), a likely consequence of limited environmental regulations in many Asian countries (Wang et al., 2014, 2021). In contrast, PFAS concentrations in Canadian and Northwest Atlantic marine mammals have declined in recent years. However, whether this is due to the implementation of strict regulations for the use and production of PFAS (Fair and Houde, 2018) or possible underestimates of PFAS exposure in marine mammals (Spaan et al., 2020) remains unclear, given only dozens of the several thousands of PFAS contaminants globally marketed are routinely monitored (Wang et al., 2021).

High concentrations of PFAS have been reported in a range of bycaught and stranded species and populations including the West

Indian manatee (*Trichechus manatus*) (Palmer et al., 2019) and the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) (Gaylard, 2017). Notably, both these examples demonstrate no detectable population effects despite elevated PFAS concentrations being reported. For example, bottlenose dolphins residing in the Swan River, Western Australia and Port River, South Australia contain some of the highest global concentrations of PFOS (up to 6975 ng/g, Stephens et al., 2019), despite both populations increasing in size over the last 30 years (Gaylard, 2017). In contrast, the Indo-Pacific humpback dolphin (*Sousa chinensis*) population in South Asia is rapidly declining at an estimated annual rate of 2.46% (Sanganyado et al., 2018). This population is chronically exposed to PFAS due to inhabiting coastal waters in the Pearl River Estuary, where they share similar trophic positions to Indo-Pacific bottlenose dolphins.

The effects of PFAS on marine mammal reproduction and development are not fully characterised (Fair and Houde, 2018). However, as revealed in hooded seals (*Cystophora cristata*) and killer whales (*Orcinus orca*), maternal transfer of contaminants is recognised as having an important role in determining the relationship between PFOS concentration and age (Andvik et al., 2021; Gabrielsen et al., 2011; Gebbink et al., 2016). High concentrations of PFAS have been reported to disrupt the endocrine system, which can lead to neurological impacts in some species (Dietz et al., 2019; Gabrielsen et al., 2011; Pedersen et al., 2015). High concentrations of PFAS (923 ng/g lipid weight) have further been linked to changes in the immune system in male walrus (*Odobenus rosmarus*) (Routti et al., 2019). Likewise, Californian sea otters (*Enhydra lutris*) have demonstrated significant infectious disease association even at modest (PFOS = 55 ng/g ww, PFOA = 60 ng/g ww) concentrations (Kannan et al., 2006). Common bottlenose dolphins (*Tursiops truncatus*) exposed to chronic concentrations (ranging between 573 and 2450 ng/g ww) of PFAS in Charleston Harbour, USA have further demonstrated increased susceptibility to disease due to resultant immunosuppression (Fair et al., 2012). However, it remains unclear if such disease results solely from PFAS contaminants or whether multiple stressors are at play. Indeed, risk characterisation of the vast number of PFAS molecules requires a cohesive research approach (Dietz et al., 2021; Wang et al., 2017).

The marine environment is undoubtedly the source of multiple stressors, acting as a sink for a wide range of anthropogenic pollutants, including trace elements, that originate from land and marine based activities. Similar to persistent organic contaminants, non-essential trace elements such as mercury and lead are proven sources for disease and reproductive failure in sentinel species (Murphy et al., 2015; Williams et al., 2021). However, while no shortage of studies on such contaminants have been conducted on marine mammals, both in the context of essential prey related inferences as well as deleterious toxicity examination (e.g., Lischka et al., 2021; Machosky-Capuska et al., 2020; Polizzi et al., 2013; Stockin et al., 2007), few have explored trace element loads in unison with emerging contaminants such as PFAS. Furthermore, given so little is presently understood about the mechanistic processes that dictate the loading of PFAS within mammalian tissue, it remains unclear what if any role trace elements play in the process, either chemically within mammalian physiology or as a context of exposure due to prey preferences. The aim of this study was to (i) characterise PFAS alongside essential and non-essential trace metal concentrations in common dolphins (*Delphinus delphis*) populating NZ waters, (2) determine potential interactions between contaminants, and (3) examine how these contaminants vary with sex, life history, and stranding location. We hypothesize life history is the primary factor dictating PFAS concentrations in NZ common dolphins examined at similar temporal and spatial scales. We further predict that some essential trace elements will be more correlated to the accumulation of PFAS loads than others, based on diet variability.

2. Materials and methods

2.1. Sample collection and storage

Tissue sampling was undertaken from 12 common dolphins involved in two independent live mass stranding events on the New Zealand coast (Table 1). Most of the carcasses ($n = 7$) were recovered from Taupō Bay, Northland ($34^{\circ} 99'S$, $173^{\circ} 71'E$) on 13 April 2019. The remainder ($n = 5$) originated from an earlier mass stranding in Okukari Bay ($41^{\circ} 20'S$, $174^{\circ} 31'E$) Marlborough Sounds on 27 February 2019. The carcasses were transported for post-mortem assessment via refrigerated transport.

Carcasses were subjectively divided into categories based on the degree of post-mortem autolysis (Table 1). Animals described as *fresh* live stranded and subsequently died on the beach immediately prior to transportation (as determined by the presence of eye moisture and absence of *rigor mortis*). *Mild* was assigned to carcasses which exhibited *rigor mortis* but which otherwise showed no external signs of decomposition. Carcasses that showed early signs of decomposition (e.g., skin discolouration and/or sloughing) were classified as *Moderate* (Stockin et al., 2009). No animals beyond moderate decomposition were considered in this study. Pathological examination and sampling were conducted according to Geraci and Lounsbury (2005). Liver samples for contaminant analyses were excised from each carcass using a stainless-steel knife and subsequently wrapped in aluminium foil prior to storage at $-20^{\circ}C$. Prior to sampling, total body length (cm) and body condition as per Stockin et al. (2009) were recorded (Table 1).

2.2. Age, reproduction and body condition

Sexual maturity was defined from histological examination of testes and gross examination of ovaries, for males and females, respectively. Sexual maturity in females was determined by the presence of at least one *corpus albicans* (CA) or *corpus luteum* (CL) on an ovary and/or they were pregnant or lactating (Murphy et al., 2009). Mature females were further categorised into four reproductive states: (1) *pregnant* (visible presence of a foetus and a CL of pregnancy but not lactating, based on the absence of milk in the mammary glands), (2) *lactating* (active mammary glands producing milk but not visibly pregnant), (3) *pregnant and lactating*, and (4) *resting* (presence of at least one CL or CA but no signs of pregnancy or lactation), as defined by Murphy et al. (2009). Teeth were used to age individual dolphins via growth layer groups (GLGs) following standard protocols. In summary, five to ten teeth of the least worn/curved teeth were extracted post-mortem from each animal and stored in 70% ethanol for further analysis. Tooth preparation methods were adapted from Lockyer (1995). Teeth were adequately fixed in formalin and decalcified in 10% formic acid. A predetermined volume of used formic acid was adjusted to neutral pH using ammonia solution and if no precipitation was noted, a volume of saturated calcium oxalate equal to that of the formic acid was added. Precipitation of any kind indicated that decalcification was not complete and immersion

in formic acid was continued. Decalcified teeth were rinsed in water for several hours (often overnight). Teeth were then mounted using OCT compound (Tissue-tek® - Sakura) onto a specimen holder and frozen to $-20^{\circ}C$ in a Leica Jung CM1800 cryotome. Sections 12–18 μm in thickness were cut along the longitudinal axis of the tooth, with the best central sections through the pulp cavity selected. These sections were then subsequently placed in pre-frozen cassettes and immediately transferred into 0.2–0.5% Toluidine blue (pH 8.4) for approximately 6 min (room temperature) before rinsed in water to remove excess stain. Finally, sections were positioned on superfrost plus slides (Menzel Glaser) and blotted dry before mounting using Entellan resinous medium (ProSciotech).

All age estimates were initially made 'blind' (i.e., with the reader having no biological information on the animal). Each section was examined twice and if there was a discrepancy between the duplicate readings, the tooth was re-examined, with knowledge of the prior estimates, until a final estimate was determined (after Westgate and Read, 2007). On occasions where ages could not be determined (i.e., in older teeth), another tooth was sectioned and read. One GLG in common dolphins is considered to represent one year of life (Gurevich et al., 1980). Body condition (Table 1) was defined as per Stockin et al. (2007).

2.3. Trace elements

The analysis of essential (iron (Fe), zinc (Zn), copper (Cu), manganese (Mn) selenium (Se)) and non-essential (mercury (Hg), nickel (Ni), cadmium (Cd), tin (Sn), antimony (Sb), lead (Pb), arsenic (As) and silver (Ag)) trace elements was undertaken byASUREQuality Laboratories, Auckland, New Zealand (Table S1). Trace elements were determined in liver by inductively-coupled plasma mass spectrometry (ICP-MS) or inductively-coupled plasma optical emission spectrometry (ICP-OES) using a Perkin Elmer NexION 300D, or 350D or 2000B and Perkin Elmer OPTIMA 7300 DV or AVIO-500, respectively. ICP-MS was used for those elements typically present at relatively low concentrations (e.g., Sn), whereas ICP-OES was used for elements that occur at higher concentrations (e.g., Cu, Zn). Approximately 1 g of hepatic tissue for each individual was digested in concentrated nitric acid with a trace of hydrofluoric acid. Full analytical quality control protocols were followed, and all metals were analysed within the laboratory's International Accreditation New Zealand (IANZ) accreditation (No. 175).

2.4. Poly- and perfluoroalkyl substances (PFAS)

2.4.1. Analytical methodology

The analysis of 33 PFAS analytes (including isomers of PFOS and PFHxS, Tables S2 and S3) in tissue samples was undertaken by ASUREQuality Laboratories, Wellington, NZ, using an electrospray ionisation liquid chromatography tandem mass spectrometry (ESI-LC-MS/MS) operated under the negative mode (Drew et al., 2021). These commercial methods have been assessed and validated by participation in

Table 1

Specimen details for common dolphins (*Delphinus delphis*) that mass stranded in Okukari Bay, Marlborough Sounds (27 February 2019) and Taupō Bay, Northland (13 April 2019). TBL = Total Body Length.

Code	Location	Sex	TBL	Age	Reproductive state	Decomposition	Body condition
KS19-10Dd	Okukari Bay, Marlborough Sounds	F	182	7	Immature	Mild	Good
KS19-11Dd	Okukari Bay, Marlborough Sounds	F	174	4	Immature	Moderate	Moderate
KS19-12Dd	Okukari Bay, Marlborough Sounds	M	201	9	Immature	Mild	Good
KS19-13Dd	Okukari Bay, Marlborough Sounds	F	189	5.5	Immature	Mild	Good
KS19-14Dd	Okukari Bay, Marlborough Sounds	F	198	10	Lactating	Mild	Good
KS19-17Dd	Taupō Bay, Northland	F	193	12	Resting	Fresh	Moderate
KS19-18Dd	Taupō Bay, Northland	M	112	0	Immature	Fresh	Good
KS19-19Dd	Taupō Bay, Northland	F	192	11	Pregnant	Fresh	Moderate
KS19-20Dd	Taupō Bay, Northland	F	189	12	Pregnant & Lactating	Fresh	Good
KS19-21Dd	Taupō Bay, Northland	F	191	15	Lactating	Fresh	Moderate
KS19-22Dd	Taupō Bay, Northland	F	197	18	Pregnant & Lactating	Fresh	Good
KS19-23Dd	Taupō Bay, Northland	F	191	7	Lactating	Fresh	Moderate

several international proficiency testing programs over several years (Drew et al., 2021). The PFAS analysis employed an isotope dilution method based on Taniyasu et al. (2005). The target analytes and internal standards are listed in Tables S2–S4. The analytical identification parameters and other method details used by the commercial laboratory were recently reported (Drew et al., 2021). Analytical standards (native target analytes and isotope-labelled internal standards) were purchased from Wellington Laboratories Inc. Guelph, Ontario, Canada. All chemicals and solvents used were of liquid chromatography–mass spectrometry (LCMS) grade or highest quality available.

The chromatographic separation was optimised to separate the linear isomers of PFHxS or PFOS from the corresponding branched isomer groups and to separate, as much as possible, the isobaric endogenous interferences from the analytes of interest. These interferences are of concern when analysing samples from mammals. Results were reported independently for the linear isomer and for two groups of branched isomers (di-methyl and mono-methyl). Total PFOS or total PFHxS represents the numerical sums of the three corresponding individual results for each compound.

2.4.2. Sample extraction

Frozen liver samples were thawed at 2–8 °C, then allowed to warm to room temperature. Samples were blended using a Waring blender to produce a homogeneous material. Approximately 1 g test portion was taken from the blended material. Test portions were fortified with internal standard. Acidified acetonitrile (2% formic acid v/v) was added, and each test portion was further homogenised using ceramic homogeniser pellets and a Merris Minimix vibrational shaker for 8 min at 50 Hz. A portion of the extract was cleaned up by dispersive solid phase extraction (dsPE) using zirconia-coated silica (Supelco Supel™ QuE Z-Sep) then solvent exchanged to 50:50 methanol/water prior to LC-MS/MS analysis. As it was not possible to obtain blank samples suitable to determine limit of detection (LOD), limit of reporting (LOR) was assigned as the equivalent to the lowest calibration standard, which was 0.5 ng/g.

2.4.3. Quality control

Quality control (QC) procedures were compliant with requirements of US DoD/DoE QSM 5.1.1 (2018). Each analysis batch included an ongoing precision and recovery (OPR) sample and an OPR blank. The OPR sample was fortified at a concentration 1–2× the method LOR. Each analysis batch also included a reagent blank (i.e., no matrix, but all the reagents and processes used in the extraction procedure). One submitted sample per analysis batch was randomly selected by the laboratory for spiking with the target analytes at the OPR concentration, before and after extraction, to assess the impact of matrix effects on the recovery of target analytes. Each analysis batch also included a randomly selected duplicate test sample.

2.5. Data analysis

Among the 33 PFAS analytes (including isomers of PFOS and PFHxS, 29 PFAS analysed), 23 compounds (Table S3) were below the LOR in all samples, and thus removed from subsequent statistical analyses. Similarly, four non-essential trace elements (nickel, lead, antimony, and tin) were removed from further statistical analyses due to their concentrations being below the LOR in all samples. Descriptive statistics were performed on all groupings relating to sex, location, and life history stage.

Statistical analyses were performed in R version 3.6.3. PFAS concentrations below the LOR were substituted by LOR/(square root of 2) for principal component analysis (PCA). Non-parametric Kruskal Wallis and Mann-Whitney tests were used to assess difference in PFAS concentrations among different categorical groups. Welch-*t*-tests, applied for pair-wise comparison to the groups, showed significant difference in PFAS concentrations. Spearman rank tests were applied to examine

correlations among PFAS, as well as between PFAS and trace elements. Minimum age was used to perform linear regression to reveal the correlation between dolphin age and the concentration of each chemical. Statistical significance was set at $p < 0.05$ for all analyses.

3. Results and discussion

3.1. Samples

A total of twelve animals were analysed from two mass stranding events in Northland ($n = 7$) and Marlborough Sounds, New Zealand ($n = 5$, Table 1). While constituting a small collective sample size, these mass stranding events offered a unique insight to PFAS levels in a pelagic species examined during relatively similar temporal (weeks apart) and spatial (<800 km) scales. Females ($n = 10$) ranged from 4 to 18 years old and from 174 to 198 cm in total body length. Seventy percent of females ($n = 7$; Table 1) were sexually mature. Of these adults, one was classified as resting mature (KS19-17Dd), one was pregnant (KS19-19Dd) and two were lactating (KS19-21Dd and KS19-23Dd). The final two mature females (KS19-20Dd and KS19-22Dd) both from Taupō Bay, were synchronously pregnant while lactating (Table 1). Both males (KS19-12Dd; 9 yr, TBL = 201 cm from Okukari Bay, Marlborough Sounds and KS19-18Dd; TBL ≤ 1 yr, 112 cm from Taupō Bay, Northland), were sexually immature (Table 1). These sex and maturity biases indicate both mass stranding events involved a nursery group of predominantly adult females, with only juvenile males present, as previously noted in mass strandings involving this species (Stockin et al., 2007; Viricel et al., 2008). Post-mortem examinations revealed decomposition concentrations were generally low; with 91.7% of carcasses either fresh ($n = 7$) or mild ($n = 4$; Table 1). All examined carcasses were classified as either in good ($n = 7$) or moderate ($n = 5$) body condition.

3.2. Profile of trace elements determined in hepatic samples

Essential Fe and Zn were the two most elevated trace elements in the analysed liver samples (Fig. 1a), with concentrations of non-essential Ni, Pb, Sb, and Sn all below LOR (Table S1). Most essential elements that serve biological functions in organisms (including Cu, Fe, Mn, and Zn), were detected in concentration ranges comparable to white-sided dolphins (*Lagenorhynchus obliquidens*) assessed in the mid-1990s in the US (Mackey et al., 1995), though considerably lower than the three species of dolphin assessed off Southeastern USA between 2012 and 2018 (Page-Karjian et al., 2020). Liver concentrations of Cu and Zn were within the range of concentrations thought to represent homeostatic control (6.3 to 12 and 37 to 148 µg/g ww, respectively (Law, 1996)). All essential elements measured in this study showed no significant difference between sexually immature or mature groups (Fig. S1).

Concentrations of Hg in the analysed livers ranged from 0.53 to 89 µg/g ww, with weaned Northland animals (i.e., excluding KS19-18Dd) ranging from 28 to 89 µg/g compared to 13 to 28 µg/g ww for weaned Marlborough Sounds animals. In both cases, the Hg:Se molar ratio in liver was less than one in almost all individuals, reflecting the successful detoxification mechanism by which organic mercury is transformed and deposited as inert mercuric selenide (Law et al., 2003). The only exception was KS19-22 Dd, the oldest female examined whose molar ratio was 1.18. At 18 years old, KS19-22Dd was simultaneously lactating while pregnant at the time of stranding in Taupō Bay, Northland.

PCA analysis revealed distinctive profiles of non-essential trace elements between the sexually immature and mature individuals (Fig. 1b). Significant differences in non-essential element loads were further revealed by the Kruskal Wallis analysis (Fig. S1). Comparatively higher concentrations of Cd (0.78 to 6.2 µg/g ww) were reported in the liver of sexually mature animals compared to immature dolphins, mostly from the stranded Marlborough group (0.029 to 1.3 µg/g ww; $p < 0.05$; Fig. S1). Similarly, the concentration of As, Hg, and Ag measured in the liver

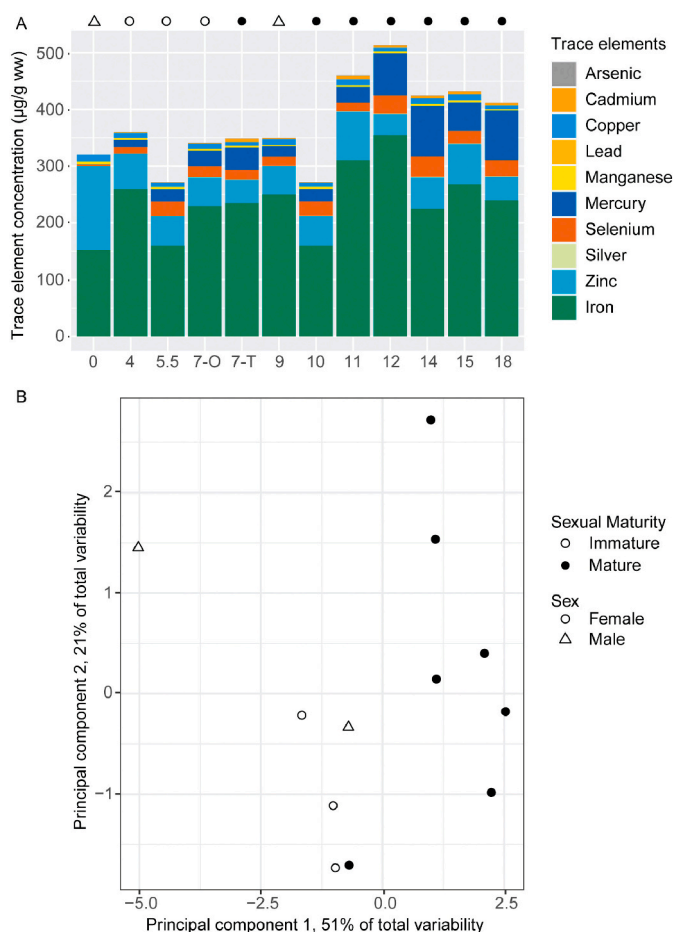


Fig. 1. Hepatic concentrations and minimum dolphin age (years) (A) and principal component analysis (PCA) (B) of trace elements detected in mass-stranded common dolphin (*Delphinus delphis*) in Okukari Bay, Marlborough Sounds (27 February 2019) and Taupō Bay, Northland (13 April 2019), New Zealand. The sex and sexual maturity of the dolphins are denoted by open (immature) or closed (mature) circles (female) and triangles (male).

samples also varied by sexual maturity ($p < 0.05$; Fig. S1). There was an interesting interaction between sexual maturity and location, though this was not discernible based on the current sample size. It should be noted that the Marlborough dolphins were primarily sexually immature, in contrast to females in Northland, who were mostly pregnant, lactating or simultaneously pregnant and lactating (Table 1). During pregnancy and lactation, females increase their nutritional and energy demands (Malinowski and Herzing, 2015), including lipid rich prey choices (Ahn et al., 2014; Young and Cockcroft, 1995). Prey consumption is known as the main source of Cd and Hg concentrations (Law et al., 2003), as well as protein and lipids to fuel basic metabolic processes in marine predators (Machovsky-Capuska and Raubenheimer, 2020). Cephalopods and fish are widely accepted sources of Cd and Hg concentrations respectively, in marine mammals (Das et al., 2003). However, anchovies are also known as a source of Cd in the diets of Franciscana (*Pontoporia blainvillei*, Polizzi et al., 2013) and common dolphins (Machovsky-Capuska et al., 2020) from South American waters.

Meynier et al. (2008) showed that common dolphins from New Zealand's North Island consumed arrow squid (*Nototodarus* spp.), jack mackerel (*Trachurus* spp.), and anchovy (*Engraulis australis*) as the most prevalent species reflected in stomach contents. Therefore, the high concentrations of Cd detected in Northland's pregnant and lactating females likely reflect the consumption of squid and anchovies within their diet. However, both the population structure (Barceló et al., 2021; Stockin et al., 2014) and wider ecology known for New Zealand

Delphinus (e.g., Dwyer et al., 2020; Hupman et al., 2018; Peters et al., 2020; Peters and Stockin, 2021) strongly demonstrate how this gregarious species span coastal seas to open ocean, with high mobility further demonstrated in genomic connectivity between New Zealand and Australian *Delphinus* (Barceló et al., 2021).

The concentrations of hepatic Hg, Se, and As observed in our study were comparable to data previously reported for this species in New Zealand (Koeman et al., 1972; Stockin et al., 2007), and the United Kingdom (Law et al., 2006). Specifically, the concentrations of Zn (4.1 to 100 µg/g ww), As (0.07 to 1.7 µg/g ww), Se (1.9 to 39 µg/g ww), and Hg (0.17 to 110 µg/g ww) in the liver of common dolphins reported by these earlier studies were also similar to those reported here. Only Cd (<0.02 to 52 µg/g ww) concentrations differed to those reported herein for New Zealand common dolphins, which may reflect age, sex and dietary differences between the sampled dolphins, or alternatively reflect differences in actual contaminant exposure between the regions.

3.3. Overall hepatic PFAS composition and concentrations

In our study, ten of the 33 PFAS analytes (including isomers of PFOS and PFHxS) in the liver samples were detected above the LOR in two or more individuals (Table S2). The total PFAS concentrations reported in all 12 hepatic samples ranged from 11.3 to 110.4 (median = 34.1) ng/g ww (Fig. 2a). These concentrations are comparable to those reported in cetacean livers in the early 2000s in Europe and South Korea (e.g., Gui et al., 2019; Hart et al., 2008; Moon et al., 2010; Rotander et al., 2012) and reflect those recently reported in harbour porpoise (*Phocoena*

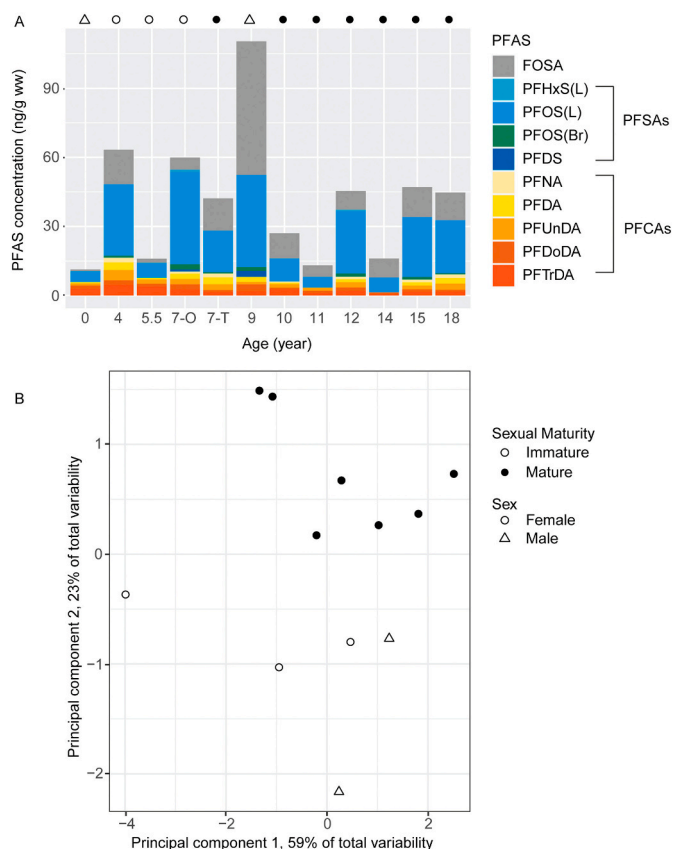


Fig. 2. Hepatic concentrations and minimum dolphin age (years) (A) and principal component analysis (PCA) (B) of PFAS detected in mass-stranded common dolphin (*Delphinus delphis*) in Okukari Bay, Marlborough Sounds (27 February 2019) and Taupō Bay, Northland (13 April 2019), New Zealand. The sex and sexual maturity of the dolphins are denoted by open (immature) or closed (mature) circles (female) and triangles (male). PFHxS(L), linear PFHxS; PFOS(L), linear PFOS; and PFOS(Br): mono(trifluoromethyl) PFOS.

phocoena) from Japan (Fujii et al., 2018). Higher hepatic PFAS have recently been reported in Mediterranean Sea common dolphin (240.3 ± 218.6 ng/g ww, $n = 2$; López-Berenguer et al., 2020) and in other cetacean species examined off the US Atlantic coast, Danish North Sea, Greenland, and South China Sea (Galatius et al., 2013; Gui et al., 2019; Lam et al., 2016; Spaan et al., 2020).

Four PFAS (i.e., linear PFOS (PFOS(L)), PFDODA, PFTrDA, and FOSA), were consistently present in the liver of all common dolphins. All dolphin livers contained PFOS(L) as the dominant PFAS (except for KS19-19Dd which exhibited slightly higher FOSA concentrations; Fig. 2a). Branched PFOS (PFOS(Br), mono(trifluoromethyl) PFOS) was detected in 7 of the 12 analysed liver samples, although accounted for only up to 5% of sum PFOS. This percentage is significantly lower than the ~17% reported within mono-isomers of PFOS produced by the 3 M company (Benskin et al., 2009). However, it is consistent with the overall low percentage of branched isomers observed in marine biota, which seem to preferentially enrich PFOS(L) (Houde et al., 2013). FOSA was the only precursor PFAS chemical detected in the analysed dolphin livers. The concentrations of sum PFOS and FOSA in the dolphin livers ranged from 4.7 to 42 ng/g ww and 0.79 to 58 ng/g ww, respectively (Table S2). These concentrations are consistent with those measured in livers from porpoises and melon-headed whales (*Peponocephala electra*) in Japan, minke whales (*Balaenoptera acutorostrata*) in Korea and beluga whales (*Delphinapterus leucas*) in Alaska (Fujii et al., 2018; Hart et al., 2008; Moon et al., 2010; Reiner et al., 2011), but considerably lower than the maximum concentrations reported in the liver of bottlenose dolphins (*Tursiops truncatus*) in South and Western Australia (up to 6975 ng/g, ww; Stephens et al., 2019) and white-beaked dolphins (*Lagenorhynchus albirostris*) in the Danish North Sea (126 to 549 ng/g ww; Galatius et al., 2013).

In addition to PFOS and FOSA, long-chain PFCAs (>C8) were also frequently detected in the livers of the examined common dolphins ($n = 7-12$). Individual PFCA concentrations are relatively low compared with PFOS and FOSA, in the range of below LOR to 4.6 ng/g. Despite the low concentrations, the characteristic ratio (i.e., >1) of odd/even per-fluorocarbon chain lengths PFCAs was observed in all liver samples, except for KS19-12Dd (a pubescent male from Marlborough Sounds) and KS19-20Dd (a pregnant, lactating female from Northland). The unique pattern in the liver of these two dolphins was characterised by the concentration of an odd chain-length PFCA being higher than its nearby even chain-length PFCA (i.e., PFNA > PFOA, PFUnDA > PFDA, PFTrDA > PFDODA). This is thought to result from oxidization of atmospheric fluorotelomer alcohol (FTOH) and has been previously observed in other marine organisms (Spaan et al., 2020).

Sum PFOS concentrations reported for females ranged from 6.5 to 42 ng/g ww and 4.7 to 27 ng/g ww for Marlborough Sounds and Northland, respectively and were not influenced by either location ($p = 0.28$) or maturity status ($p = 0.39$). FOSA ranged from 1.8 to 15 ng/g ww and 5 to 14 ng/g ww for Marlborough Sounds and Northland, respectively. Neither location ($p = 0.41$) or sexual maturity ($p = 0.61$) affected FOSA concentrations.

FOSA:PFOS ratios reported here were in the range of 0.12 to 1.38 (mean = 0.65; Fig. S2), and comparable to the previously reported range of 0.01 to 1.28 in cetaceans in the Northern Hemisphere (Spaan et al., 2020). The lowest FOSA:PFOS ratio measured in a liver sample (0.12; KS19-10Dd) related to a physically mature (total body length = 182 cm) female who remained sexually immature (Table 1). The FOSA:PFOS ratios observed in cetaceans are generally higher than in other marine mammals and are likely attributable to the inability of cetaceans to transform FOSA to PFOS (Dassuncao et al., 2018).

Interestingly, although shorter-chain PFAAs (e.g., PFPeA, PFHxA, PFHpA, PFBS, and PFHxS) have been detected in hepatic samples from northern hemisphere cetaceans (Fujii et al., 2018; Gui et al., 2019; Lam et al., 2016; Spaan et al., 2020), only linear PFHxS were detected in two common dolphins sampled in this study. The reason for the absence of these short-chain PFAAs is unclear, which warrants future studies to

investigate these short-chain PFAAs and their precursors present in the aquatic environment and trophic food webs of cetacean habitats. This will enable us to understand their potential impact on marine mammals in New Zealand, and furthermore assess potential health risks for humans associated with cultural harvesting (i.e., ingestion) of cetaceans, as determined within a One Health framework (Backer et al., 2019; Bossart and Duignan, 2018; Movalli et al., 2018).

Although the overall profile of PFAS in the analysed liver sample did not vary significantly by location, body condition and/or reproduction state, PCA analysis did indicate that unique PFCA profiles might be related to sexual maturity (Fig. 2b, Fig. S3). Kruskal Wallis analyses suggest this is likely driven by the presence of PFDODA and PFTrDA, long-chain (C₁₂, C₁₃). Congeners, respectively, that exhibited higher concentrations in the liver of immature dolphins (Fig. S4). Approximately, a 1.5 fold higher concentration of PFDODA or PFTrDA was measured in the livers of the immature versus the mature group that primarily consisted of lactating and/or pregnant females except for one resting female. The lower concentrations of these compounds add in the livers of immature animals may be related to the dietary and metabolic traits of lactating and/or pregnant females. Insignificant differences were observed among the specimens at different reproduction states ($p > 0.1$). The lack of association of PFAS concentrations with reproduction state in this study may be related to our limited sample size. Alternatively, our findings may indicate differences in the diets, nutritional requirements and metabolic characteristics of individual animals, which may be dominating and obscuring observed correlations.

Bioaccumulation of PFAS in marine mammal is typically influenced by species, age, gender, size, and habitat (Fair and Houde, 2018; Tartu et al., 2018). Higher concentrations of PFAS have been detected in apex predators, such as polar bears (*Ursus maritimus*), compared to lower trophic species such as fur seal pups (*Callorhinus ursinus*) (Berger et al., 2004; Kannan et al., 2001). Clearly, the trophic position and feeding behavior of animals are both important when determining the concentrations and potential effects of PFAS (Borga et al., 2004; Kannan et al., 2006). Indeed, trophic transfer has been identified as an important exposure route to biota (Tomy et al., 2004).

3.4. PFAS and trace element concentrations relative to dolphin ages

The chemical profile analyses revealed differences in the concentrations of certain PFAS and trace elements by life history of the stranded dolphins. We therefore further examined the age and concentration relationships for PFAS and trace elements. The estimated age of the 12 stranded dolphins ranged from 0 to 18 years. Significant correlations were identified between ages and five contaminants, including PFTrDA and four non-essential trace elements, i.e., Hg, Ag, Cd, and Se ($p < 0.05$; Fig. 3).

PFTrDA is the only PFAS that demonstrated significant correlation with dolphin age, with concentration decreasing significantly with increasing age ($p < 0.05$; Fig. 3a). Conversely, Hg, Ag, Cd, and Se all demonstrated significant positive-correlations with age (Fig. 3b–e). Diet and maternal transfer through gestation and/or lactation are the two major exposure pathways for trace elements in cetaceans (Bossart, 2010). The negative and positive correlation of PFTrDA and four trace elements, respectively indicate different bioaccumulation/bio-magnification mechanisms for these contaminants in the cetacean life cycle.

3.5. Correlations among hepatic PFAS and trace elements

To gain an insight into the sources of PFAS and both essential and non-essential trace elements, we performed Spearman correlation analyses on all chemicals measured in the hepatic samples. Significant positive-correlations were observed within respective PFAS or trace element groups. Between several PFAS and trace elements, only negative correlations appear significant, suggesting potential different

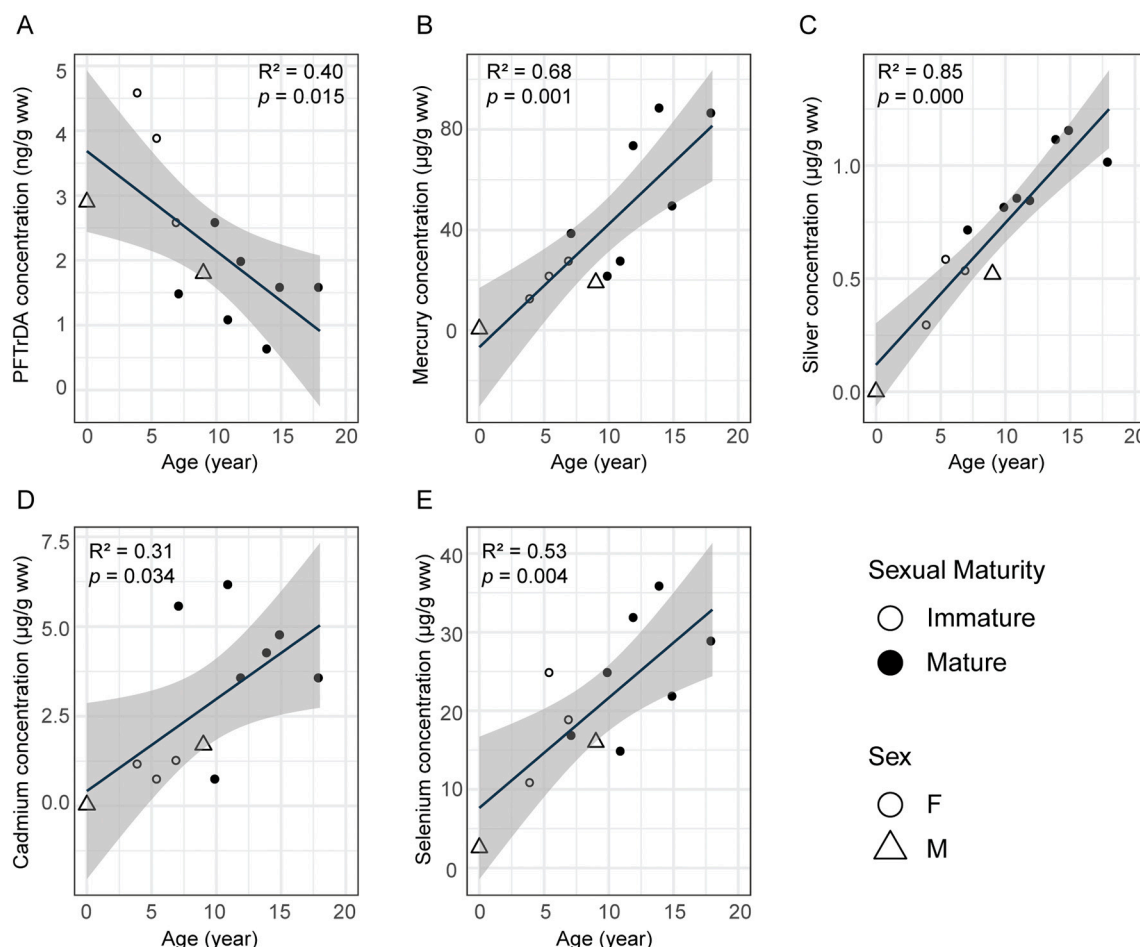


Fig. 3. Linear regression analysis between age (years) and hepatic chemical concentration in common dolphins. The grey shade represents the confidence interval of the regression. Only chemicals demonstrated statistically significant correlation ($p < 0.05$) with age are shown.

exposure pathways for these different classes of anthropogenic contaminants (Fig. 4). Most notably, the age-related PFTrDA demonstrated insignificant correlation with all other PFAS but significant negative correlation with the four trace elements that are also related to age.

Not all PFAS detected in the liver samples have significant positive correlations with each other, indicating the dolphins could have been exposed to different sources of these compounds (Fig. 4). FOSA is significantly correlated to PFOS and PFDA, indicating a potential common source of these compounds (Fig. 4). Interestingly, PFOS also demonstrated significant positive-correlation with other PFAS that lack statistical correlation with FOSA, indicating PFOS might originate from multiple sources (Fig. 4). We also note that Spearman's correlation measures only monotonic relationships, so we cannot rule out more complex interactions or dynamic equilibria.

3.6. Potential sources of PFAS in New Zealand

The PFAS profiles identified in our study composed mostly of long-chain PFAAs. In New Zealand, PFOS and PFOA were excluded from the Firefighting Chemicals Group Standard in 2006, with the import and usage of PFOS further restricted in 2011. The production and importation of PFOA as a stand-alone compound is additionally regulated, though there currently is no information on the import and usage of other PFAS substances in New Zealand.

While historically there is also very little information on environmental sources of PFAS in New Zealand (Coakley et al., 2018), typical point sources of PFAS in the environment could include industry (PFAS

manufacturers and/or users), and sites where intensive firefighting training occurs (e.g., military bases and international and regional airports) have been identified. Industrial sources are considered limited with no PFAS manufacturer existing currently within New Zealand nor Australia. Recent investigations have assessed potential accumulation of PFAS in biota and sediment in Waitemata Harbour, Auckland in response to historical use and storage of aqueous film forming foams (AFFFs) at the Devonport Naval Base and Whenuapai Air Force Base (Pattie Delamore, 2019). Across four investigations and multiple sites, PFAS levels above LOR were reported across invertebrates, fish, sediment and surface water, with PFOS the most commonly detected PFAS chemical across the sites. PFOS concentrations in all five surface water sites at Whenuapai exceeded the 95% ecosystem protection guidelines (HEPA, 2018). Furthermore, PFAS concentrations in the sediment collected at Devonport Naval Base Sea Safety Training Squadron outfalls are the highest recorded to date in sediment in New Zealand (Pattie Delamore, 2019).

Other sources of PFAS discussed during the recently completed all government national response to PFAS in New Zealand included surveillance fire-fighting sites including airports, urban and agriculture runoff, landfill leachates, and industrial and municipal wastewater effluents (Rumsby and Manning, 2018). Indeed, landfill leachate and wastewater treatment (e.g., discharge of contaminated water, application of contaminated biosolids) are anticipated to be a major point source of PFAS contamination based on international studies (Coggan et al., 2019; Gallen et al., 2018), although wastewater monitoring for PFAS in New Zealand to date has been relatively minimal (Rumsby and

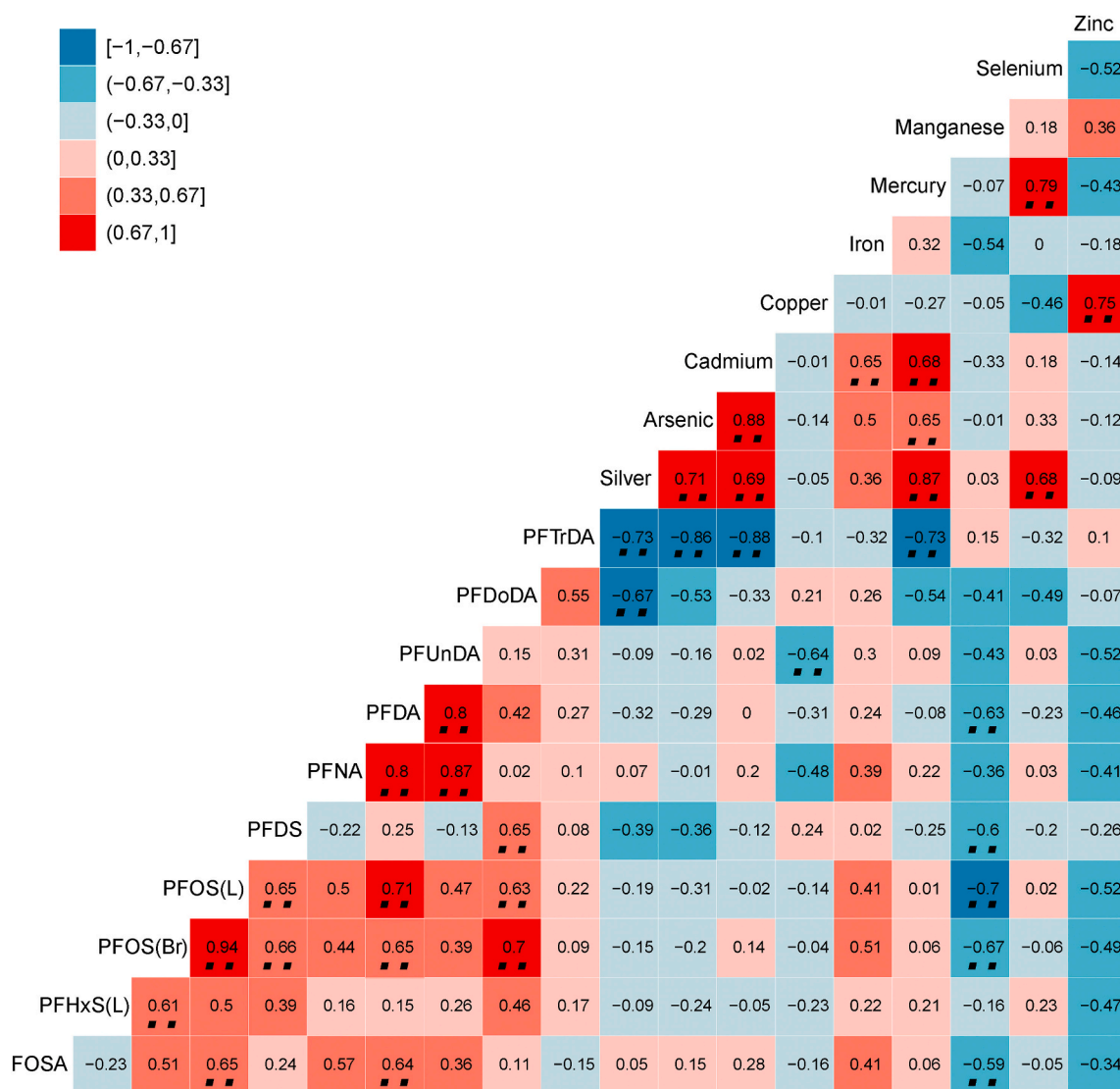


Fig. 4. Spearman correlation matrix for PFAS measured in common dolphin livers. The red and blue intensities indicate the positive or negative correlation, and numbers indicate correlation coefficients. Statistically significant correlations are denoted by dots ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Manning, 2018). Finally, it would be worthwhile investigating imports from countries still producing and using PFAS in large quantities as some products may contain PFAS, intentionally or otherwise.

4. Conclusions

PFAS concentrations reported in this study for common dolphins in New Zealand are comparable to those measured in other marine mammals from more industrial regions of the globe. This warrants investigation given PFAS is neither manufactured in New Zealand nor considered widely prevalent within the terrestrial environment. While variability between the orders and species of marine mammal remain a possible explanation, sources of PFAS chemicals recorded here within New Zealand common dolphins remain unidentified. The data confirm the persistence, global distribution, and bioaccumulation potential of these molecules. While hepatic PFAS profiles generally did not vary by location, body condition and/or reproductive state in common dolphins examined in this study, PFCA concentrations did appear to be influenced by sexual maturity. Furthermore, significant negative correlations were observed between the concentration of a few PFAS (i.e., FOSA, PFDoDA, PFTrDA, PFUnDA, PFDA, PFDS, and PFOS), and trace elements in the

liver of dolphins with different reproductive states. This finding indicates further research is required to elucidate the relationship between these different classes of anthropogenic contaminants. Future emphasis should also be placed on the examination of PFAS burdens of individuals within the context of historical pregnancy rates, using ovarian scarring as a proxy. Additional studies are also warranted to investigate the short-chain PFAAs and their precursors, specifically their presence within the aquatic trophic food webs. To our knowledge, this study represents the first peer reviewed data of PFAS concentrations in cetaceans from South Pacific Oceania waters, and the first to assess PFAS in the context of trace elements concentrations. As such, presented analyses provide significant insight into the PFAS global distribution and their potential to impact to marine wildlife.

CCRediT authorship contribution statement

K.A. Stockin: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing. **S. Yi:** Data curation, Formal analysis, Writing – original draft, Writing - review & editing. **G.L. Northcott:** Conceptualization, Funding acquisition, Writing – review &

editing. **E.L. Betty:** Data curation, Funding acquisition, Writing – review & editing. **G.E. Machovsky-Capuska:** Formal analysis, Writing – review & editing. **B. Jones:** Formal analysis, Funding acquisition, Writing – review & editing. **M.R. Perrott:** Formal analysis, Funding acquisition, Writing – review & editing. **A. Rumsby:** Conceptualization, Data curation, Funding acquisition, Writing – review & editing. **M.A. Thelen:** Formal analysis, Writing – review & editing. **L. Graham:** Data curation, Writing – review & editing. **E.I. Palmer:** Data curation, Project administration, Writing – review & editing. **L.A. Tremblay:** Conceptualization, Data curation, Funding acquisition, Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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