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Environmental Toxicology

Correlations between per- and polyfluoroalkyl substances (PFASs) and body morphometrics in fledgling shearwaters impacted by plastic consumption from a remote Pacific island.

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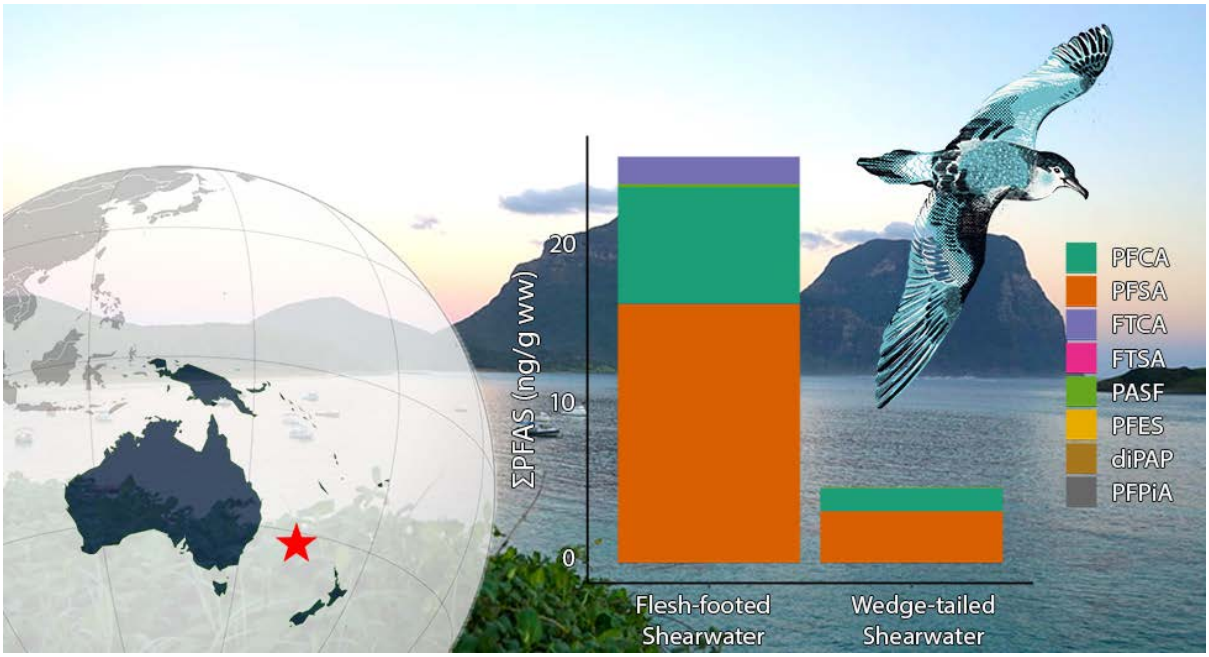
Abstract: This study investigated the concentrations of 45 per- and polyfluoroalkyl substances (PFASs) in fledgling Flesh-footed Shearwater (*Ardenna carneipes*; n = 33) and Wedge-tailed Shearwater (*A. pacifica*; n = 9) livers via LC-MS/MS and their relationship to body morphometrics and ingested plastic mass recorded in 2019 on Lord Howe Island, NSW, Australia. Sixteen PFASs were detected, of which perfluorooctanesulfonate (PFOS) was the dominant compound, detected in 100% of birds (1.34 to 13.4 ng/g ww). Long-chain perfluorocarboxylic acids, including perfluorodecanoic acid (PFDA; <0.04 to 0.79 ng/g ww) and perfluorotridecanoic acid (PFTrDA; <0.05. to 1.6 ng/g ww) were detected in >50% of birds. There was a positive correlation between PFDA and PFTrDA concentrations and wing chord length (R_s=0.36, p=0.0204; R_s=0.44, p=0.0037 respectively), and between PFDA concentrations and total body mass (R_s=0.33, p=0.032) suggesting that these

compounds may impact shearwater fledgling morphometrics. Plastic was present in the intestinal tract of 79% of individuals (<7.6 g), although there was no correlation between PFAS concentrations and plastic mass, indicating ingested plastic is not the likely primary exposure source. The widespread occurrence of PFASs in fledgling marine birds from a relatively pristine location in the southern hemisphere suggests that further studies in adult shearwaters and other marine birds are warranted to investigate whether there are any long-term physiological effects on bird species.

Graphical Abstract



Keywords: per- and polyfluoroalkyl substances (PFASs); persistent organic pollutants (POPs); avian toxicity; marine plastics; contaminants of emerging concern

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INTRODUCTION

Similar to the persistent organic pollutants (POPs) of the mid- to late-20th century, per- and polyfluoroalkyl substances (PFASs) are an emerging class of semi-volatile and non-volatile anthropogenic contaminants that may pose a threat to seabirds throughout the world, due to their capacity for long-range transport in the atmosphere and oceans (Armitage 2009). In particular, oceanic transport plays an important role in the fate of non-volatile compounds, as these compounds can bioaccumulate and biomagnify in biota, potentially leading to detrimental impacts (Butt 2010). Furthermore, as plastics continue to accumulate in the oceans and are mistaken for

food by marine birds (Battisti 2019), the role of plastic ingestion as a vector for organic pollutants exposure, including PFASs, is not yet known.

PFASs are a family of over 4700 compounds (OECD 2018), many of which have been used widely as surfactants since the 1950s in aqueous-film-forming-foam (AFFF), non-stick cookware and weather-proof textiles (Buck 2012). PFASs are generally categorised according to their functional group and named by the number of carbon atoms. For example, perfluorocarboxylic acids (PFCAs; $CF_3(CF_2)_xCOOH$) and perfluorosulfonic acids (PFSAs; $CF_3(CF_2)_xSO_3H$) are the most widely studied PFAS groups, as they are the terminal, "forever" products of many PFAS precursors (Wang 2017). Notably, three of these compounds have had their use restricted or banned by the United Nations Environmental Programme (UNEP) under the Stockholm Convention due to their persistence, bioaccumulation and toxicity in biota: perfluorooctanesulfonic acid (PFOS; $C_8F_{17}SO_3H$), perfluorooctanoic acid (PFOA; $C_8F_{15}COOH$) and perfluorohexanesulfonic acid (PFHxS; $C_6F_{13}SO_3H$) (UNEP/POPS/POPRC.6; UNEP/POPS/POPRC.12; UNEP/POPS/POPRC.15). Polyfluoroalkyl substances include precursor and/or intermediate compounds that contain one or more CH_2 moiety in the aliphatic chain and are increasingly replacing regulated compounds. Consequently, novel PFASs such as fluorotelomer carboxylic acids (FTCAs) and perfluoroalkyl sulfonyl fluorides (PASFs) are being increasingly detected in the environment where they may pose a potential toxicological risk (Barzen-Hanson 2017; Strynar 2015). A potential source of FTCAs and PASFs may be due to the biotransformation of semi-volatile PFASs, such as n:2 fluorotelomer alcohols (n:2 FTOHs) (Butt 2014), that are distributed via long-range atmospheric transport (Armitage 2009).

Flesh-footed Shearwaters (*Ardenna carneipes*; FFSH) and Wedge-tailed Shearwaters (*A. pacifica;* WTSH) are marine seabirds from the family Procellariidae. They are distributed in the Pacific and Indian Oceans, with the former also having a range in the Southern Ocean south of Australia (BirdLife International 2018; 2019). Flesh-footed Shearwaters are categorised as 'Near-Threatened' by the International Union for Conservation of Nature (IUCN), whilst WTSH are listed as species of 'Least Concern' (BirdLife International 2018; 2019). Similar to many seabird species globally, both FFSH and WTSH have decreasing population trends (Croxall 2012; Dias 2019; Gorta 2019), despite localised areas of variation (Bancroft 2004). Seabirds face many anthropogenic threats such as bycatch, habitat loss, competition for resources and solid waste pollution, i.e. plastic debris (Dias 2019). Due to the large foraging area and high trophic order of seabirds (Mallory 2010), they can be ideal biomonitoring species for understanding the impacts in the world's oceans, due to pollution (Braune 2005), fish stock declines (Frederiksen 2007) and climate change (Thompson 2001).

Concentrations of PFASs have mainly been studied in juvenile birds from the northern hemisphere, in areas such as the Arctic region that are exposed due to long-range transport, and near fluorochemical facilities that represent a greater exposure to birds (Table 1). The blood, egg and liver are the ideal tissues to sample as they tend to have the highest concentration of PFASs relative to the other organs (Custer 2019; Custer 2012; Gebbink 2012). However, plasma and eggs are more often sampled due to their non-destructive nature and the ability for longitudinal studies (Leat 2013; Route 2014). PFOS is the most detected and usually the most abundant compound

found in juvenile bird livers (Table 1) and plasma (Table S4). Typically, birds have higher PFASs body burdens adjacent to localised point-sources when compared to those from remote regions where long-range transport is the primary source (Table 1). Juvenile birds hatched in remote regions have PFOS concentrations up to 188 ng/mL in plasma (Sletten 2016). However, near fluorochemical manufacturing facilities, PFOS concentrations that are orders of magnitude higher are reported (35,624 ng/mL in plasma), which decrease as a function of distance from the facilities (Lopez-Antia 2019).

The length of the CF_2 chain is the primary driver in determining the fate of these compounds in the environment. Sediment sorption and bioaccumulation potential can be predicted by the octanol to water partition coefficient (K_{OW}), which increases with CF_2 chain length (Higgins 2006). Short-chain PFCAs ($C_n < 8$) and PFSAs ($C_n < 7$) are found less frequently above detection limits in birds (Sturm 2010), however long-chain PFCAs ($C_n > 7$) and PFSAs ($C_n > 6$) (Buck 2011) will tend to bioaccumulate in marine and terrestrial organisms and are reported to biomagnify along trophic gradients (Barghi 2018; Ng 2014). Odd-chained PFCAs are typically found in greater concentrations compared to their even-chained homologues (e.g., PFNA > PFOA, PFUDA > PFDA and PFTrDA > PFDoDA) (Bossi 2015), which may be evidence of the degradation of FTCAs, although this effect has not been observed in avian species (Butt 2014). Recently, replacement compounds and precursor PFASs are frequently being detected in adult bird populations (Eriksson 2016; Letcher 2015). The consequent degradation to terminal perfluoroalkyl acids (PFAAs) and ecotoxicological impact of replacement compounds could pose a potential risk to vulnerable species and the viability of their offspring.

Concentrations of PFASs in juvenile birds may be due to residual exposure from maternal transfer from adult to egg (Newsted 2007). However, the contribution of PFASs in the egg from the mother vary widely between individuals (Gebbink 2012) and will change as a function of the egg-laying order (Lasters 2019), although as Procellariforms invariably lay only one egg, the latter may not be a factor. Overall, diet is the primary pathway of PFAS exposure in juvenile birds after they are hatched (Custer 2019; Custer 2012; Letcher 2010). The contribution of atmospheric exposure of PFASs in birds has been reported (Gewurtz 2016), but few studies have shown concentrations of volatile PFASs and their potential metabolites (Eriksson 2016; Guruge 2011). Previous reports have found that plastic can be found in up to 90% of fledgling shearwater individuals, averaging 2.7 ± 10.6 g plastic per bird (n = 38) (Lavers 2014). Marine plastic debris may transport and concentrate trace elements and some organic contaminant concentrations from the environment (Bond 2011; Tanaka 2019) and can act as a vector for their bioaccumulation into birds (Tanaka 2020). Despite reports that PFASs will also sorb to the surfaces of marine plastic (Llorca 2014), there is no empirical data on the contribution of PFAS to individuals due to plastic exposure in birds, unlike hydrophobic POPs, in which marine plastic is a known vector for body burden contamination (Li 2016).

The estimated median lethal dose (LD_{50}) of PFOS in juvenile quail and duck are between 61 and 150 mg/kg bw/day (Newsted 2005), orders-of-magnitude greater than the typical environmental concentrations found around the world (Ahrens 2011; Xiao 2017). In the North Atlantic Ocean, for example, PFOS concentrations range between 8.6 – 36 pg/L (Yamashita 2008), well below the LD_{50} value. The sub-lethal effects of

chronic exposure to PFASs, such as physiological (Costantini 2019), immunological (Sletten 2016), reproductive (Blévin 2017), biochemical (Lopez-Antia 2017), and genetic (Nakayama 2008) impacts, have been used to assess the risk of the compounds in the environment. Due to the highly proteinaphillic nature of some PFASs (Jones 2003), other sub-lethal effects have been measured in biomonitoring studies such as negative correlation with serum protein (Lopez-Antia 2017), cholesterol (Hoff 2005), alanine aminotransferase, lactate dehydrogenase and creatinine kinase (Peden-Adams 2009). Furthermore, there is evidence that PFOS will negatively affect the expression of genes involved in carbohydrate transport and metabolism; and intracellular trafficking, secretion, vesicular transport (Nakayama 2008). To assess the risk of PFASs to bird species, the concentrations of each compound must be accurately quantified in individuals from a wide range of species.

The current study reports the occurrence of 45 PFASs in two species of juvenile pelagic seabirds that died as a result of beach-wash and road-kill, from the remote South Pacific Lord Howe Island. Correlations between PFAS concentrations, body morphometrics and plastic ingestion were compared to determine potential ecotoxicological impacts on juvenile shearwaters. To the authors' knowledge, this is the first study of the occurrence of PFASs in livers of pelagic seabirds from the Southern Hemisphere.

METHODOLOGY

Sample Collection and Morphometry

The study was conducted on Lord Howe Island, New South Wales, Australia (31°33'15" S, 159°05'06" E) with the permission of the Lord Howe Island Board (LHIB07/18), New South Wales Office of Environment and Heritage (SL100169), Victorian Department of Environment, Land, Planning and Water (10009019), University of Tasmania Animal Ethics (A18480), Department of Primary Industries, Parks, Water and Environment (DPIPWE), and the Australian Bird and Bat Banding Scheme (ABBBS). Samples were taken from dead fledglings (80 to 90 days old) of two species of shearwater found beach-washed and as road-kill, collected between 27 April and 9 May 2019 and autopsied within one hour of recovery. Fledglings tend to take flight just before sunrise so fresh specimens were sampled each morning within hours of death. Thirty-three Flesh-footed Shearwaters (*Ardenna carneipes*) and nine Wedge-tailed Shearwaters (*A. pacifica*) were sampled in total. The following body morphometric measurements were made: body mass (± 10 g), wing chord (± 1 mm), culmen and head + bill length (± 0.1 mm). The proventriculus and gizzard were examined, with the mass of plastic (± 0.0001 g) were recorded for each bird (Table S2). The livers of these birds were immediately excised and collected in LDPE zip-lock bags and stored at -20 ℃ until analysis

Chemicals and reagents

Hypergrade acetonitrile (ACN, 75-05-8), hypergrade methanol (MeOH, 67-56-1), ammonium acetate (> 99.99, 631-61-8) were purchased from Merck Millipore (Victoria, Australia). Type I ultrapure water was obtained from reverse osmosis (RO) water coupled with MilliQ Reference A+ system (18.2Ω; TOC < 5 ppm, Merck, Victoria, Australia). Magnesium sulphate/sodium acetate QuEChERS packets (7.5 g); and C18/primary secondary amine (PSA) dSPE sorbent (100 mg) were obtained from

Agilent Technologies (Victoria, Australia). Primary PFAS standards (n = 45) and mass-labelled surrogates (n = 14) were obtained individually from Wellington Laboratories (Ontario, Canada). Naming conventions for PFASs are according to Buck (2011). A complete list of PFASs, their long-form names and CAS numbers are available in Table S1. In brief, eleven PFCAs (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUDA, PFDoDA, PFTrDA and PFTeDA), nine PFSAs (PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoDS and PFECHS), as well as several novel classes of PFASs including four fluorotelomer sulfonic acids (FTSAs: 4:2 FTSA, 6:2 FTSA, 8:2 FTSA and 10:2 FTSA), three fluorotelomer carboxylic acids (FTCAs: 3:3 FTCA, 5:3 FTCA and 7:3 FTCA), eight perfluoroalkane sulfonyl fluorides (PASFs: FOSA, EtFOSA, MeFOSA, FOSAA, EtFOSAA, MeFOSAA, EtFOSE and MeFOSE), three perfluoroalkyl phosphinic acids (PFPiAs: 6:6 PFPiA, 6:8 PFPiA and 8:8 PFPiA), four fluorotelomer phosphate diesters (diPAPs: 6:2 diPAP, 6:2/8:2 diPAP, 8:2 diPAP and diSAmPAP) and three perfluoro ether-based substances (6:2 Cl-PFESA, 8:2 Cl-PFESA and ADONA).

Per- and polyfluoroalkyl substance extraction

Whole livers were weighed and transferred to 15 mL polypropylene centrifuge tubes, homogenised with a rotor-stator dispersal tool for approximately one minute (PT1200E, Polytron) (n = 42). The extraction method was adapted from Baduel (2015) and was conducted in batches of no more than 18 samples at a time and included the two QAQC samples (see Section 2.4). Briefly, approximately 25 ng of mass-labelled PFAS internal standard (Table S1) were added before the addition of 5 mL ACN before the samples were vortexed and sonicated for 30 minutes, respectively. Approximately 0.75 g magnesium sulphate and 0.25g sodium acetate were added before the samples were centrifuged at room temperature (3392 *g*, 10 minutes). Approximately 1 mL of supernatant was added to a vial containing 50 mg each of C18 and PSA for the removal of large organics such as sugars, lipids and sterols. The extract was then briefly vortexed and centrifuged (2000 *g*, 5 minutes) and the resulting supernatant (250 µL) was then transferred to a polypropylene autosampling vial with a polypropylene cap for mass spectrometry analysis.

LC-MS/MS analysis

An adapted analytical procedure was performed, as previously detailed (Coggan 2019). In brief, the analysis was performed on an Agilent 1290 Infinity II liquid chromatography system coupled with an Agilent 6495C triple quadrupole mass spectrometer. Chromatographic separation was achieved in a 15-minute run using a 50 mm C18 Zorbax Eclipse column using 2 mM ammonium acetate aqueous phase and 100% MeOH organic phase. The following gradient of MeOH: t_0 = 10%, $t_{0.5}$ = 10%, $t_{2.5}$ = 55%, t_9 = 90%, $t_{9.1}$ = 100%, $t_{11.5}$ = 100%, $t_{11.6}$ = 10% was used. The source conditions are as follows: drying gas temperature and flow = 250 °C at 11 L/min; sheath gas temperature and flow = 375 °C at 11 L/min; nebuliser pressure = 25 psi; capillary and nozzle voltage = 2500 and 1500 V; and iFunnel high and low-pressure RF = 90 and 60 V. Transitions and collision energies were optimised for each compound and a summary can be found in Table S1. A ten-point calibration curve was used to quantify the concentrations of each analyte ranging between 0.05 and 50 ng/mL ($R^2 > 0.99$) whilst mass-labelled concentrations remained constant in calibration levels at 5 ng/mL in MeOH.

Quality assurance and quality control (QAQC)

The quality of acquired LC-MS/MS data was verified by the addition of a laboratory control sample (LCS) and method blank (MB), one of each was included with the corresponding batch of a maximum of 18 samples. The LCS and MB were prepared following the same procedure as the samples, except without liver. The LCS was spiked with 25 ng native PFAS mix to measure the recovery of each compound. The average internal standard response from the samples and QAQC were compared to the average internal standard response from the calibration curve. A detailed report on QAQC results can be found in Figure S2. Briefly, internal standard recoveries for each mass-labelled compound fell between 50 and 150%. The internal standard corrected recoveries for LCS fell between 70 and 130 % and the concentration of PFASs in the MB fell below the method detection limit for each compound.

The method reporting limit (MRL) was defined by the lowest calibration level for compounds with S/N > 3:1. Concentrations of PFASs in samples had to meet the following conditions for quantification: (1) S/N > 10:1, (2) ISTD recovery response between 50% and 150%, (3) concentration within the calibration range, (4) retention time within 5% of highest calibration result, (5) qualifying ion ratio (where available) within 20% of highest calibration result. Results that did not meet one or more of these conditions were treated as <MRL.

Statistical analyses

Data were acquired and quantitated using Agilent MassHunter Workstation and Quantitative Analysis 10.1 respectively. Descriptive statistics, statistical analysis and data visualisation were performed with R v4.0.2 (R Core Team 2017) and RStudio (1.2.5019, Boston, Massachusetts, USA) with tidyverse v1.3.0.9000 (Wickham 2019), ggplot2 v3.3.2 (Wickham 2016), rstatix v0.6.0 (Kassambara 2020) and psych v2.0.7 (Revelle 2019) packages. Statistical analysis was based on those described by Robuck (2020) and the results of tests were reported according to NHST guidelines (Dushoff 2019; Erickson 2020). Measurements of body morphometry, plastic mass and concentrations of PFASs were checked for normality using the Shapiro-Wilk test before analysis, where normally distributed data results in $p > 0.05$. All morphometric measurements, plastic mass, as well as PFOS, PFTrDA and $\sum_{45}$PFAS concentrations were determined to be non-normal and were $\log_{10}$-transformed resulting in normal distribution. Geometric mean and 95% confidence intervals (CI) were calculated for concentrations of each PFAS using MRL/2 for concentrations <MRL and zero for non-detects when the frequency of detection was >50%. MANOVA and Cohen's d-test for effect size was used to test differences in body morphometry and total plastic mass between species. Further statistical analysis of censored data were tested using non-parametric tests. Mann-Whitney-Wilcoxon U test was used to test for differences in concentrations of PFASs. Principal component analysis (PCA) and Spearman's correlation matrix (R_s) were calculated for morphometric measurements, plastic mass and PFAS concentrations. The level of significance was set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Flesh-footed and Wedge-tailed Shearwater morphology & ingested plastic

Flesh-footed shearwaters (FFSH) had increased body morphometry compared to WTSH (Global MANOVA: p = 0.0000, df = 36; Figure 1). The total body mass of FFSH (mean: 281 g, 95% CI: 210 – 434 g) was greater than WTSH (mean: 205 g, 95% CI: 170 – 278 g) (p = 0.0018, Cohen's d = 1.07). The wing chord lengths of FFSH (mean: 255 mm, 95% CI: 231 – 304 mm) were also greater than WTSH (mean: 231 mm, 95% CI: 212 – 260 mm) (p = 0.0002, Cohen's d = 1.56). Finally, the head + bill length and culmen lengths in FFSH (mean: 94 mm, 95% CI: 88 – 99 mm and mean: 42 mm, 95% CI: 39 – 45 mm respectively) were greater than those of WTSH (mean: 81 mm, 95% CI: 77 – 84 mm and mean: 36 mm, 95% CI: 32 – 40 mm respectively) (p = 0.0000, Cohen's d = 4.18; p = 0.0000, Cohen's d = 2.26). Each of the morphometric measurements were highly and significantly correlated with one another ($R_s > 0.36$, $p < 0.01$; Table S3) The average mass of liver excised from each bird was 1.69 g (95% CI: 1.48 – 1.70 g). Plastic was found in the intestinal tract of 79% of shearwaters, with no statistically clear differences in the incidence between species ($p > 0.05$; Figure 1). The total mass of plastic found in FFSH and WTSH intestinal tract were 0.346 g (95% CI: 0 – 0.58 g) and 0.149 g (95% CI: 0 – 0.69 g) respectively. The mass of plastic found in shearwaters from 2019 is in the same range as those found previously from these populations on Lord Howe Island by Lavers (2014) in 2011 (range: 0 – 64.1 g) and Puskic (2019) in 2017 (range: 0 – 27.4625 g). The full details of the morphometric measurements and plastic masses are presented in the Supporting Information.

Number and concentration of per- and polyfluoroalkyl substances (PFASs)

Of the forty-five compounds analysed, sixteen PFASs from five classes (PFSA, PFCA, FTCA, PASF and PFPiA) were detected in all juvenile shearwater livers (n = 42). The $\sum_{45}$PFASs ranged from 2.08 to 21.51 ng/g ww in shearwater livers and concentrations did not appear different between species ($p > 0.05$). In order of decreasing concentration, PFOS, PFNA, PFUDA, PFTrDA and PFDA were detected in over 50% of birds (Figure 2). Liver PFOS concentration (geometric mean: 3.44 ng/g ww; 95% CI: 1.5 to 8.5 ng/g ww) was more than six-fold greater than the next most abundant compound. PFNA was detected in 98% of shearwaters with a mean concentration of 0.58 ng/g ww (95% CI: 0.37 to 0.98 ng/g ww). PFUDA was detected in 95% of shearwater livers with a mean concentration of 0.55 ng/g ww (95% CI: 0.24 to 0.96 ng/g ww). PFTrDA was detected in 94% of FFSH (n = 33) and 78% of WTSH (n = 9) with average concentrations of 0.41 ng/g ww (95% CI: 0.19 – 0.69 ng/g ww) and 0.19 ng/g ww (95% CI: 0.02 – 0.42 ng/g ww), respectively. PFDA was detected in 90% of shearwater livers with a mean concentration of 0.32 ng/g ww (95% CI: 0.02 to 0.72 ng/g ww), which was significantly less than the concentration of PFUDA (p = 0.0000). Eleven compounds with detection frequencies less than 50% were not subject to further analysis, particularly from the FTSA, PFESA/PFECA, and diPAP classes, of which none were detected.

There was no statistically clear difference in mean concentrations when comparing the PFOS, PFNA, PFDA and PFUDA between FFSH and WTSH fledglings from Lord Howe Island ($p > 0.05$, Table 2) and as such, the two species will be discussed

together. Furthermore, despite reported differences in foraging areas between the two species (Miller 2018; Reid 2012), similar concentrations of PFASs in these species suggests that the environmental concentrations of these compounds surrounding Lord Howe Island are relatively homogenous. By contrast, concentrations of PFTrDA were estimated to be 0.17 ng/g ww (95% CI: 0.08 to 0.29 ng/g ww) greater in juvenile FFSH compared to WTSH (Mann-Whitney-Wilcoxon U = 259, p = 0.0007 two-tailed; Table 2). While there is a real difference in PFTrDA concentrations between species, the difference is relatively small and negligible.

PFOS is the most frequently detected and most abundant compound in marine and terrestrial juvenile birds in biomonitoring studies and data from the current study are consistent with previous findings (Table 1). Adult shearwaters will travel approximately 400 km on average from the nest to feed their offspring (Alonso 2012). PFOS has a half-life in birds of 230 days (Tarazona 2015), meaning that PFOS concentrations measured in the nestling shearwaters on Lord Howe Island are representative of the environmental concentrations within the feeding radius.

Of the PFCAs, the mean concentration of compounds with C>9 decreased from PFUDA > PFNA > PFTrDA > PFDA (Table 2). Long odd-chain PFCAs are reported in the literature with greater frequency at high concentrations compared to their next shorter, even-chain moiety (i.e., PFNA > PFOA, PFUDA > PFDA and PFTrDA > PFDoDA) (Braune 2013; Chu 2015; Eriksson 2016; Gebbink 2012; Gewurtz 2016). PFOA (range: <0.05 to 0.07 ng/g ww) and PFDoDA (range: <0.05 to 0.45 ng/g ww) were detected in 2% and 38% of juvenile shearwater livers from Lord Howe Island respectively, which is consistent with previous findings (Table 1). In a review of PFAS concentrations in a range of biota, including humans, PFOA is frequently detected in mammals and fish, however, it is not frequently detected in birds, and as such, the absence of PFOA in this study is supported by the literature (Sturm 2010). The observed PFCA trends in shearwaters may be due to C11 and C13 chemistries have greater global emissions compared to the C10 and C12 respectively (Armitage 2009), and the biomagnification potential for PFNA is greater than PFOA (Haukås 2007; Kelly 2009; Tomy 2004).

Saturated FTCAs, such as 7:3 FTCA, are transformation products of precursor PFAS such as FTOHs. FTCAs were previously detected in the liver and eggs of terrestrial bird-of-prey species (Order: accipiteriiformes and falconiiformes) from Japan and Sweden ranging between 6.8 and 62 ng/g ww and <0.24 and 2.7 ng/g ww, respectively. 7:3 FTCA was also detected in nearshore species, such as swans and mallards to a lesser extent (Eriksson 2016; Guruge 2011). 7:3 FTCA was detected above the method detection limit (MDL = 0.28 ng/g ww) in two birds, at 0.64 and 4.5 ng/g ww, respectively. This is the first time this compound has been reported in birds from the Southern hemisphere. Precursor compounds, such as n:2 FTOHs, are known to degrade in the atmosphere to odd-numbered PFCAs, such as PFNA (Ellis 2004). There is evidence to suggest that odd-chained compounds are the result of preferred biotransformation pathway of precursor compounds that occur in higher trophic organisms by α-oxidation of n:3 FTCAs, an intermediate degradation product of the volatile n:2 FTOHs (Butt 2014). This indicates that atmospheric PFAS emission may play a role in the contamination of these birds.

6:6 PFPiA was detected in a single FFSH at 0.19 ng/g ww (Table 2), the first reported detection of this class of compound found in marine birds. Whereas, 6:8 PFPiA and 8:8 PFPiA were not detected in any shearwater livers. To the authors' knowledge, PFPiAs have only been detected in one previous study. 6:6 PFPiA was detected in the blood of cormorants from the continental USA averaging in concentration between 0.20 and 1.6 ng/g ww, where these compounds are used as surfactants in pesticide sprays for agricultural use (De Silva 2016). The ecotoxicological effects of PFPiAs are not known for birds, however, they are suspected endocrine disruptors in zebrafish (Liu 2019) and there is evidence they biotransform to terminal PFAAs after exposure (Lee 2012). EtFOSE was detected in both FFSH and WTSH (12% and 11% respectively) (Table 2). No other PASFs were detected in shearwater livers in this study. To the authors' knowledge, this is the first reported detection of EtFOSE in birds.

In this study, PFAS concentrations found in juvenile shearwaters are generally lower than those reported for birds impacted by long-range transport and manufacturing plants in the Northern Hemisphere (Table 1), except for Golden Eagles from Norway which had a maximum concentration of $\sum_{10}$PFAS = 1.2 ng/mL in plasma (Sonne 2010). In concordance with this study, marine seabirds from a remote island in the Southern Indian Ocean had similarly low concentrations of PFASs compared to Northern Hemisphere counterparts, with average ∑PFAA concentrations ranging between 1.7 and 7.2 ng/g ww between three species of tern (van der Schyff 2020). Based on the much lower exposure of PFASs in shearwaters from this study, it is likely the southern Pacific Ocean surrounding the remote Lord Howe Island is less contaminated than the oceans of the Northern Hemisphere.

Correlation with body morphometrics

The difference in mass, head-to-bill and culmen lengths were clustered along the first and second principal components, representing 69.9% of the data (Figure S1). The influence of the body morphometrics and the PFAS were separated along the second principal component. The effects of PFOS, PFNA, PFDA, PFUDA and PFTrDA were clustered along the first and second principal components (Figure S1) and significantly correlated with each other ($R_s > 0.42$, $p < 0.001$; Table S3), indicating a possible common source of each of these compounds or similar chemical kinetics that would result in comparable uptake and depuration rates. Furthermore, Puskic (2019) found no relationship between fatty acid composition and body condition of shearwaters previously sampled on Lord Howe Island in 2017.

Concentrations of PFDA and PFTrDA were weakly positively correlated with wing chord length ($R_s > 0.36$, $p < 0.05$; Figure 3). Furthermore, PFDA was weakly correlated with body mass ($R_s = 0.33$, $p = 0.032$; Figure 3). Overall this suggests a possible effect of increased size of body morphometry from PFCA exposure. Tartu (2014) reported negative correlations with PFTrDA and corticosterone concentrations in plasma from adult Black-legged Kittiwakes (*Rissa tridactyla*) exposed to PFASs by long-range transport, concluding that there is a potential for long-chain PFCAs to have a negative impact on overall body condition. PFASs, such as PFTrDA, may modulate the action or availability of hormones in birds, due to their ability to disrupt transport proteins in serum (Jones 2003). Decreased concentrations of glucocorticoids, such as cortisol and corticosterone, in birds positively influence the

growth of various organs, including the muscle, spleen, testis, bursa and overall body weight (Hull 2007). Conversely, physiological effects of PFASs have not been reported in other juvenile birds (Løseth 2018), nor in birds with significantly greater exposure to PFASs (Custer 2019).

Effect of plastic ingestion

There was no statistically clear correlation between the concentrations of each PFAS or total PFASs and the mass of plastic found in each bird's proventriculus, gizzard or total intestinal tract (Table S3). Determination of the concentration of the sorbed PFAS was not possible as the plastic was characterised using conventional visual analytical techniques (microscope; fourier transform infrared spectroscopy, FTIR) which would have resulted in PFAS contamination. It is also difficult to predict the fate of PFASs that have been exposed to the warm acidic conditions of the gastrointestinal tract. Nonetheless, PFASs were found sorbed to plastic pellets and sediment collected from beaches in the Mediterranean Sea up to 115 ng/kg (Llorca 2014). Despite the evidence of PFAS plastics sorption, there is limited information on the potential of plastics to act as vectors for PFAS exposure and the subsequent effects of organisms that ingest plastics. The PFASs detected in the juvenile shearwaters are likely introduced and accumulated from the fish, squid and other foodstuffs foraged by the adults. Diet is the primary pathway for PFAS exposure to juvenile birds (Gómez-Ramírez 2017), and PFASs, such as PFOS and long-chain PFCAs, are known to biomagnify in marine seabirds (Barghi 2018).

Strengths and limitations

This study presents the concentrations of a large suite of PFASs, including novel and emerging classes and products of atmospheric deposition, not well described in the literature. Further studies should be directed to the potential exposure of semi-volatile PFASs such as n:2 FTOHs to investigate the pathways of biotransformation in birds. As the investigation into exposure and impacts of PFASs continues globally, attention needs to be paid to long-chain PFCAs and n:2 FTCAs, which are being increasingly detected in avian species. Furthermore, the pathways of exposure from semi-volatile PFASs, such as n:2 FTOHs, should be investigated as a source of PFCA and PFSA exposure by way of metabolism and biotransformation.

The ability to detect concentrations of PFASs below 1 ng/g has allowed the authors to understand the potential impacts of long-chain PFCAs without the hindrance of excessive censored data. The trace level detection allowed the examination of correlations between PFASs and body morphometrics, despite the relatively small sample size in this study. Ex-situ studies on the sub-lethal effects of long-chain PFCAs are needed to confirm the apparent trend as there is a lack of data in this field. The developing fields of metabolomics and genomics will also be crucial to understanding the potential sub-lethal effects of PFASs to birds and other wildlife. The authors also acknowledge the potential impact of co-contaminants that were not reported in this study that may also contribute to the body morphology of the birds.

The absence of many compounds over several classes of PFASs provides information to policymakers for the management of these compounds in the future. The continued study into the fate of PFASs in birds impacted from known sources from the southern hemisphere is also warranted, given that AFFF impacted sites have resulted in highly

contaminated avifauna (Sharp 2020). Without studies on the PFAS exposure to the adult shearwater population on Lord Howe Island or the contamination present in the food-web, it is difficult to speculate to the source precisely. Furthermore, while a correlation between plastics ingestion and PFAS was not observed in this study, it should be noted that only macroplastics (>1 mm) were recorded and further research should also include smaller micro- (<1 mm) and nanoplastics (<1 □m).

CONCLUSION

This was the first study to quantify a range of PFASs in juvenile marine seabirds (n = 42) from the southern hemisphere impacted by long-range transport. Of 45 PFAS measured, 17 were detected in the liver of FFSW (n = 33) and six compounds were detected in livers of WTSH (n = 9) from the relatively pristine location of Lord Howe Island, Australia. PFOS was detected with the greatest frequency and abundance, in agreement with previous studies. There was no statistically clear difference between concentrations of PFASs in FFSH and WTSH. Long-chain PFCAs, particularly carbon lengths between 9 and 13 were also detected in >50% of birds, albeit in lower concentrations compared with PFOS. Concentrations of these compounds were highly and significantly correlated, suggesting a common source of exposure (likely via diet or to a lesser extent, maternal transfer). Notably, the current study provides evidence of increased body mass and length in individuals with greater concentrations of PFDA and PFTrDA. The significance of increased body morphometry is unclear and continued monitoring of this population is recommended. 7:3 FTCA, EtFOSA, EtFOSE and 6:6 PFPiA were detected in few individuals, indicating exposure to novel classes of PFASs that may degrade into stable PFCAs or PFSAs. Finally, there was no clear relationship between the mass of macroplastics found in the digestive tract and concentrations of detectable PFASs. As such, plastic ingestion is not likely contributing to the exposure of juvenile birds to PFASs, rather they are being exposed by other means, such as diet or maternal transfer.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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(such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Figure 1: Body morphometry results from Flesh-footed Shearwaters (FFSH; n = 33, Green boxplots) and Wedge-tailed Shearwaters (WTSH; n = 9; Orange boxplots) collected from Lord Howe Island, 2019: A) total mass of individuals (p = 0.0017), B) head + bill (HB, p = 0.0002) length, C) wing chord (WC, p = 0.0000) length, D) culmen (Cul. p = 0.0000) length, and E) total plastic mass (p = 0.6585). Global MANOVA: p = 0.0000.

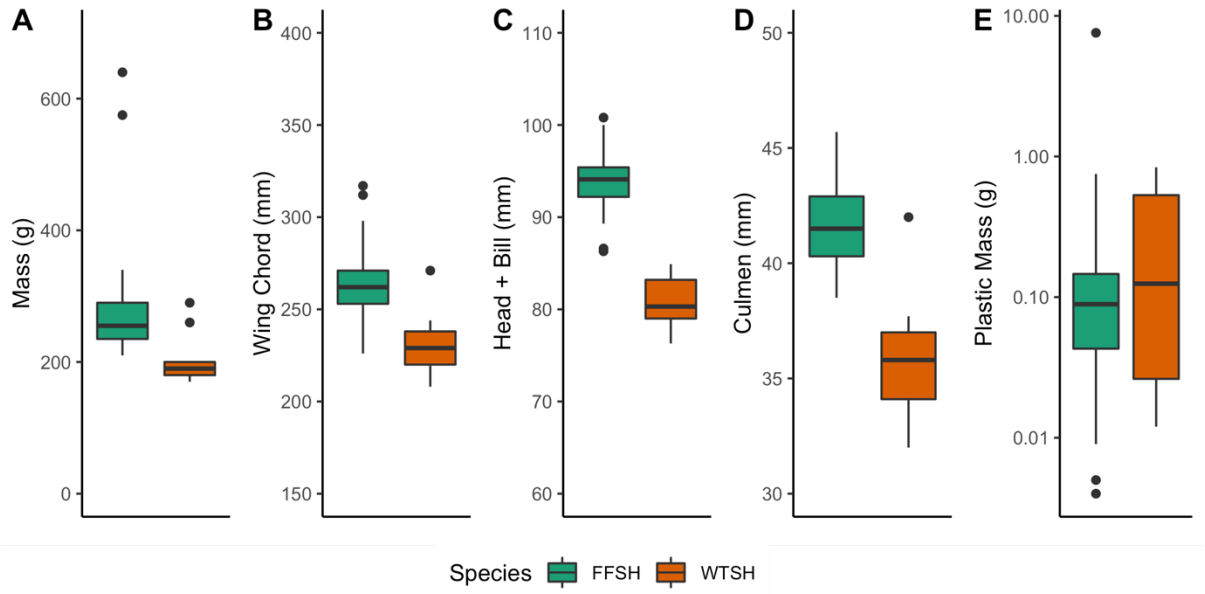

Figure 1

Figure 2: Concentration (ng/g ww) of A) $\sum_{45}$PFAS and PFOS; and B) PFNA, PFDA, PFUDA and PFTrDA in Flesh-footed Shearwater (FFSH, n = 33, Green boxplots) and Wedge-tailed Shearwater (WTSH; n = 9, Orange boxplots) liver from Lord Howe Island in April to May 2019.

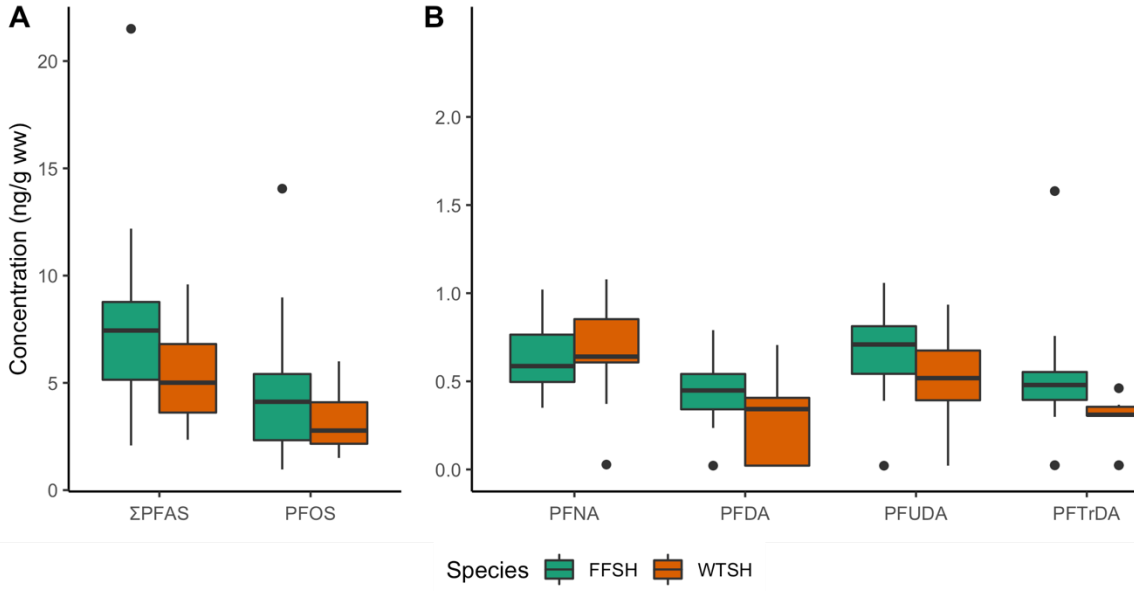

Figure 2

Figure 3: Scatterplot of PFAS concentrations and log_{10}-transformed morphometric measurements in Flesh-footed Shearwaters and Wedge-tailed Shearwaters (n = 42) from Lord Howe Island in 2019. A) PFDA (blue) and body mass, B) PFDA, log_{10}PFTrDA (yellow) and wing chord length. The dotted line represents the method reporting limit (MRL). R_s and p-values derived from Spearman's rank correlation.

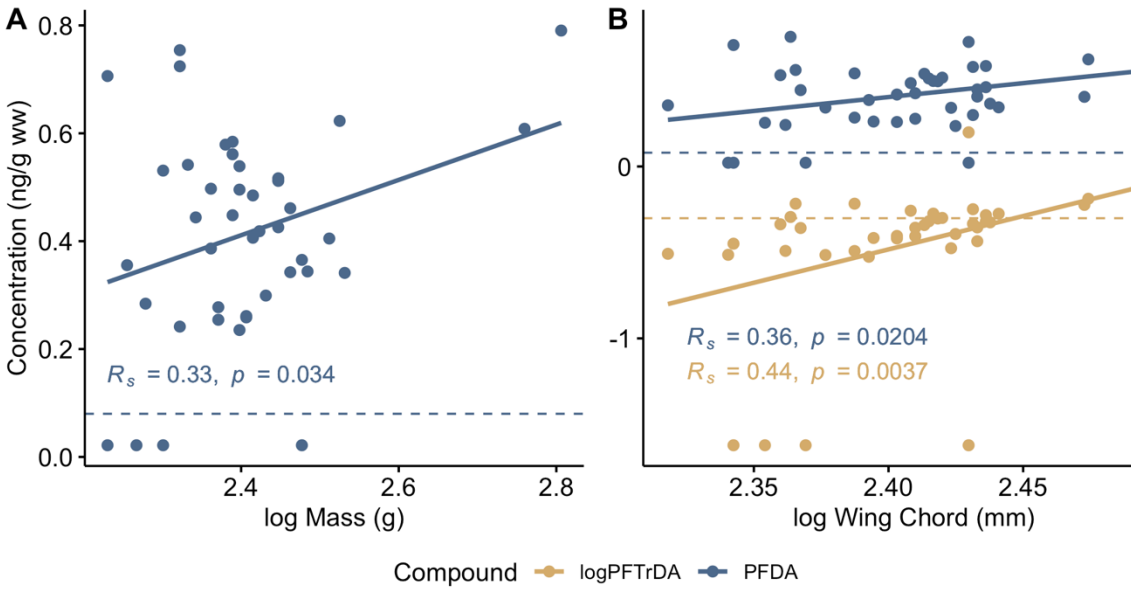

Figure 3

Table 1: Summary of reported concentration ranges of PFOS, PFNA, PFDA, PFUDA, PFTrDA and ∑PFAS in juvenile bird livers from field biomonitoring studies. Concentrations in ng/g ww, unless stated otherwise.

Country	Family	Common name	n	PFOS	PFNA	PFDA	PFUDA	PFTrDA	Reference
Long-range transport									
Australia	Procellariidae	Flesh-footed Shearwater	33	0.96 – 14.1	<0.06–1.02	<0.04 – 0.79	<0.04 – 1.06	<0.05 – 1.58	This study
Australia	Procellariidae	Wedge-tailed Shearwater	9	1.50 – 6.0	<0.06–1.08	<0.04 – 0.71	<0.04 – 0.94	<0.05 – 0.46	This study
Domestic									
USA	Laridae	Herring Gull	10	16.49 – 255.9	0.7 – 2.5	1.14 – 4.03	0.54 – 4.97	<1.0 – 6.4	Robuck (2020)

USA	Procellariidae	Great Shearwater	10	11.14 – 249.4	0.6 – 3.5	0.49 – 5.16	3.31 – 8.84	<1.0	Robuck (2020)
South Korea	Laridae	Black-tailed Gull	8	34.2 – 2510	0.73 – 4.61	1.65 – 5.04	6.67 – 10.1	4.46 – 9.70	Barghi (2018)
Manufacturing									
USA	Laridae	Royal Tern	6	98.45 – 242.8	4.7 – 8.7	14.78 – 29.46	8.77 – 14.69	<1.0 – 2.7	Robuck (2020)
USA	Laridae	Sandwich Tern	2	208.6 – 279.9	9.9 – 9.9	32.18 – 32.98	15.07 – 18.48	<1.0	Robuck (2020)
USA	Laridae	Laughing Gull	1	114.31	6.00	17.5	4.72	<1.0	Robuck (2020)
USA	Pelecanidae	Brown Pelican	2	46.05 – 46.71	3 – 3.3	10.94 – 11.95	5.95 – 6.6	<1.0	Robuck (2020)
USA	Hirundinidae	Tree Swallow	24	<9.9 – 107	NR	NR	NR	NR	Custer (2012)
USA	Hirundinidae	Tree Swallow	10	166 – 265	<0.64 – 0.8	n.d.	<0.64– 1.40	NR	Custer (2019)
Belgium	Paridae	Eurasian Blue Tit	35	69 – 3323	NR	NR	NR	NR	Hoff (2005)
Belgium	Paridae	Great Tit	48	17 – 2788	NR	NR	NR	NR	Hoff (2005)

Table 2: Summary of detection frequency, geometric mean concentrations, (95% confidence interval) and [range] (ng/g ww) of PFASs found above detection limits in juvenile Flesh-footed Shearwater (FFSH; n = 33) and Wedge-tailed Shearwater (WTSH; n = 9) from Lord Howe Island in 2019. Note: MRL/2 was substituted for values below the Method Reporting Limit.

Compound	Flesh-footed Shearwater (n = 33)		Wedge-tailed Shearwater (n = 9)		Mann-Whitney U-test
PFBS	6%	- [<0.05 - 0.2]	0%	n.d.	
PFHxS	30%	- [<0.05 - 0.63]	0%	n.d.	
PFHpS	12%	- [<0.05 - 0.22]	0%	n.d.	
PFOS	100%	3.6 (1.34 – 8.75) [0.96 - 14.05]	100%	2.93 (1.52 – 5.43) [1.5 - 6]	W = 181 p = 0.3266
PFPeA	3%	- [<0.11 - 0.34]	0%	n.d.	
PFHpA	6%	- [<0.05 - 0.17]	0%	n.d.	
PFOA	3%	- [<0.05 - 0.07]	0%	n.d.	
PFNA	100%	0.61 (0.39 – 0.92) [0.35 - 1.02]	89%	0.48 (0.17 – 1.04) [<0.06 - 1.08]	W = 132 p = 0.6238
PFDA	97%	0.40	67%	0.16	W = 203.5

		(0.24 – 0.74)		(0.02 – 0.64)	p = 0.0947
		[<0.04 - 0.79]		[<0.04 - 0.71]	
PFUDA	97%	0.62	89%	0.37	W = 203.5
		(0.41 – 0.97)		(0.11 – 0.92)	p = 0.0948
		[<0.04 - 1.06]		[<0.04 - 0.94]	
PFDoDA	48%	-	0%	n.d.	
		[<0.05 - 0.45]			
PFTrDA	94%	0.41	78%	0.19	W = 259
		(0.19 – 0.69)		(0.02 – 0.42)	**p = 0.0007**
		[<0.05 - 1.58]		[<0.05 - 0.46]	
PFTeDA	18%	-	0%	n.d.	
		[<0.05 - 0.25]			
EtFOSE	12%	-	11%	-	
		[<0.08 - 0.4]		[<0.08 - 0.48]	
7:3 FTCA	6%	-	0%	n.d.	
		[<0.28 - 4.51]			
6:6 PFPiA	3%	-	0%	n.d.	
		[<0.06 - 0.19]			