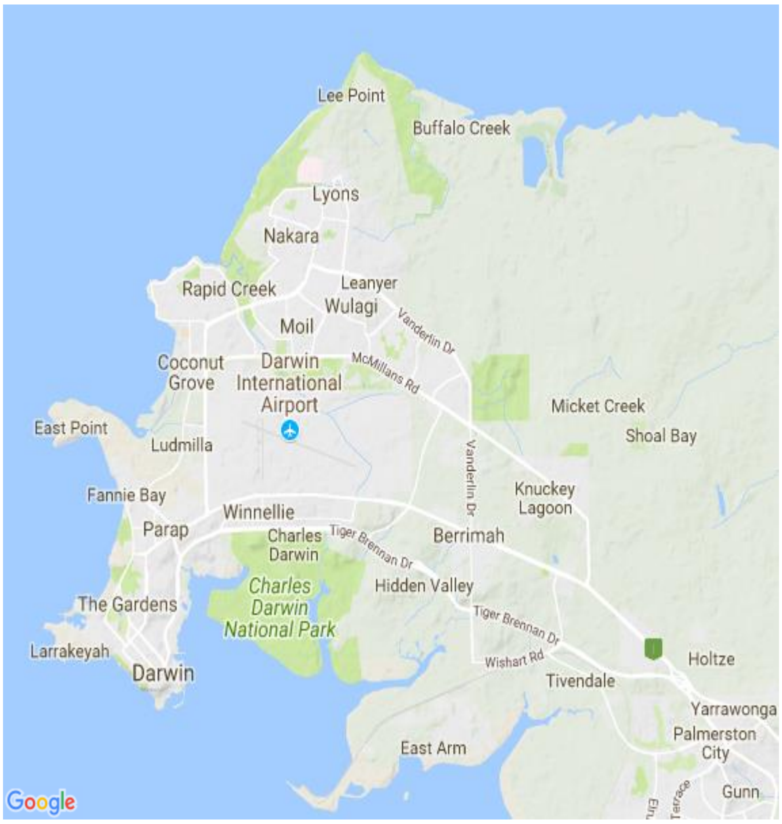
Per - and Polyfluoroalkyl Substances (PFAS) testing in aquatic foods from Darwin Harbour

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Prepared for Northern Territory Department of Health

Soumini Vijayasarathy, Sharon Grant, Jennifer Braeunig, Linus Mueller, Sarit Kaserzon, Jochen F. Mueller

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1. Introduction

Per- and poly-fluoroalkyl substances (PFAS) are a group of synthetic chemicals that have been used since the 1950s in several household and industrial products, such as non-stick cookware, mist suppressants, and fire-fighting foams. Despite their commercially favourable properties, PFAS have become of concern to human and environmental health due to their persistence in the environment, long-range transport, potential toxicological effects and tendency to bioaccumulate [1-3].

Until recently, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), two of the most common PFAS, were included in certain types of fire-fighting foam. Many countries have now phased out or are in the process of phasing out the use of PFOS and PFOA. However, investigations on historical use of fire-fighting foams that contained PFAS have shown that these chemicals have the potential to be transported through the soil to contaminate surface and ground water [4, 5].

In April 2016, a PFAS interagency working group (PFASIWG) was established in the Northern Territory (NT) to implement a co-ordinated approach to the investigation and response to potential environmental and health issues related to PFAS. As part of the NT PFAS Legacy Site Investigation strategy developed by PFASIWG, the Department of Health (DoH) and Northern Territory Environmental Protection Authority (NT EPA), along with other government agencies, initiated the investigation into the presence of PFAS in water and soils at locations that may have historically used large quantities of PFAS-based products such as areas around airports and firefighting training facilities. Darwin International Airport (DIA) lies within several creek catchments, including Rapid, Ludmilla, Reichardt, Sadgroves and Bleesers Creeks. Of these, Rapid Creek catchment and Ludmilla Creek catchment (to the north-west) form a large part of the DIA site. Preliminary investigations identified the presence of PFAS at various sites along Rapid Creek and Ludmilla Creek, as a precautionary measure DoH issued an advisory to the general public recommending against the consumption of foods from these creeks until further study on PFAS levels in aquatic foods was completed.

In 2016, the DoH commissioned Charles Darwin University (CDU) and The University of Queensland (UQ) to undertake a study on PFAS levels in sediment and aquatic food species commonly harvested by indigenous people including several species of mollusc, termed 'traditional' aquatic foods, from Rapid Creek and Ludmilla Creek and a reference (background) site Fright Point. Whilst PFAS levels in traditional aquatic food from Ludmilla and Rapid Creek were up to two orders of magnitude higher than samples collected from Fright Point, overall it was concluded that minimal risk was expected from eating aquatic food based on the median (most likely) consumption levels [6].

UQ were commissioned to extend the study to investigate PFAS levels in different species of fish and crustaceans caught opportunistically from Rapid, Ludmilla and Bleesers Creek (a third investigation site, lying 15 km south-east of the airport). The aim of this project was to estimate dietary exposure in relation to new health based guidance values for PFAS released by the Food Standards Australia New Zealand (FSANZ) [7]. The PFAS considered in this dietary exposure assessment were those for which guidance values are currently available, i.e. PFOS, perfluorohexane sulfonate (PFHxS) and PFOA.

The main objectives of this study were:

1) To quantify PFAS levels in aquatic food from PFAS impacted waterways within the catchment area around the Darwin International Airport, and
2) Assess dietary exposure of PFAS in different aquatic foods and estimate tolerable daily intake (TDI) for aquatic foods from contaminated sites in relation to the new TDI levels defined by FSANZ [7]

2. Sampling Methods

2.1. Sample collection

Samples were provided by the Department of Infrastructure, Planning and Logistics (DIPL), from three locations; Rapid Creek, Ludmilla Creek and Bleesers Creek (Figure 1). In total, three species were collected at undefined locations within Bleesers Creek (golden snapper (n = 5), catfish (n = 6) and mudcrabs (n = 3)); one species from Rapid Creek (mudcrabs, n = 9); and 4 species from Ludmilla Creek (mudcrabs (n = 9), speckled shrimp (n = 14) and giant tiger prawn (n = 1) from the boat ramp; barramundi (n = 8) from the lower length of Ludmilla Creek). Details of the sample location are listed under Appendix A.

Sample handling was managed by Department of Primary Industry and Resources. Liver samples and fillets were collected from fish using a stainless steel knife and transferred into a zip lock bag with tweezers. Mudcrab hepatopancreas were transferred into zip lock bags whole. For all samples, the knife and tweezers were rinsed with water between samples. All aquatic tissue samples were placed in separate double zip lock bags and kept on ice until they were received at QAEHS, where the samples were stored in a -20 °C freezer until sample analysis.

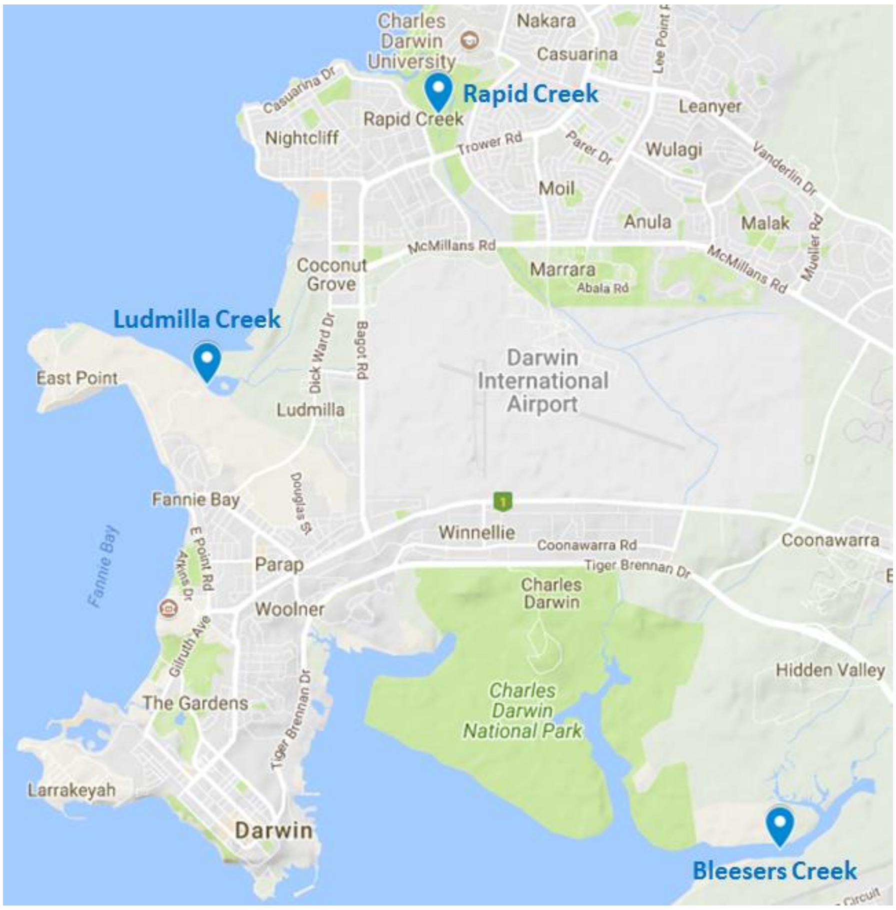

Figure 1 : Three sampling sites; Rapid Creek, Ludmilla Creek and Bleesers Creek

2.2. Protocol for PFAS analysis

Due to the limited number and amount of specimen for each species, samples were combined based on location into composite samples (Table 1). Composites of golden snapper muscle, mudcrab muscle, catfish muscle and liver from Bleesers Creek; mudcrab muscle from Rapid Creek; prawn muscle, mudcrab muscle, barramundi muscle and liver from Ludmilla Creek were analysed for 13 PFAS (see Appendix B for the list of analytes). Also analysed were composites of mudcrab hepatopancreas, for which the exact sampling location was not recorded and were considered to be from either Rapid or Ludmilla Creek. More detailed composite information (e.g. sampling date and individual specimen size) is given in Appendix A. All samples were wiped with methanol to avoid external PFAS contamination before extraction. Equal weights (2.0 - 3.0 g) of tissue were taken from each sample and homogenized with a mortar and pestle.

Table 1: List of composites prepared for PFAS analysis; M – muscle, L – liver, H – hepatopancreas

Composite ID	Sample type	n*	Location
	MUSCLE		
SNAPM_BC1	Golden snapper	2	Bleesers Creek
SNAPM_BC2	Golden snapper	3	Bleesers Creek
CATM_BC1	Catfish	3	Bleesers Creek
CATM_BC2	Catfish	3	Bleesers Creek
CATL_BC1	Catfish	3	Bleesers Creek
CATL_BC2	Catfish	3	Bleesers Creek
MUDM_BC1	Mudcrab	3	Bleesers Creek
MUDM_RC1	Mudcrab	3	Rapid Creek
MUDM_RC2	Mudcrab	3	Rapid Creek
MUDM_RC3	Mudcrab	3	Rapid Creek
MUDM_LD1	Mudcrab	3	Ludmilla Creek
MUDM_LD2	Mudcrab	2	Ludmilla Creek
BARRAM_LD1	Barramundi	4	Ludmilla Creek
BARRAM_LD2	Barramundi	4	Ludmilla Creek
PRAWM_LD1	Prawn	5	Ludmilla Creek
PRAWM_LD2	Prawn	3	Ludmilla Creek
PRAWM_LD3	Prawn	3	Ludmilla Creek
PRAWM_LD4	Prawn	2	Ludmilla Creek
PRAWM_LD5	Prawn	1	Ludmilla Creek
	LIVER/HEPATOPANCREAS		
CATL_BC1	Catfish	3	Bleesers Creek
CATL_BC2	Catfish	3	Bleesers Creek
BARRAL_LD1	Barramundi	4	Ludmilla Creek
BARRAL_LD2	Barramundi	4	Ludmilla Creek
MUDH_R/LD1	Mudcrab	4	Rapid/Ludmilla
MUDH_R/LD2	Mudcrab	3	Rapid/Ludmilla

* -number of samples in each composite

Extraction and clean up:

1.0 g of homogenate was weighed into 15 mL polypropylene centrifuge tube and spiked with nine surrogate mass labelled PFAS. Samples were digested with 200 mM NaOH/methanol and extracted twice with acetonitrile in an ultrasonication bath. Extraction was followed by clean up on Bond Elut Carbon cartridges (100 mg). Eluates were blown down to 400 µL under a gentle nitrogen stream at 40 °C and brought up to 1.0 mL with 5.0 mM aqueous ammonium acetate. Internal standards ^{13}C8-PFOS and ^{13}C8-PFOA were spiked into each sample to account for volume correction and compensate for instrument drift.

Instrument analysis:

Analysis was performed using high performance liquid chromatography (Nexera HPLC, Shimadzu Corp., Kyoto, Japan) coupled to a tandem mass spectrometer (QTrap 5500 Ab-Sciex, Concord, Ontario, Canada) operating in negative electrospray ionisation mode, and using multiple reaction monitoring (MRM) mode.

5 µL was injected onto a Gemini NX C18 Column (50 × 2 mm, 3 um, 110 Å) at 50 °C. A pre column (C18, 50 × 4.6 mm, 5 µm) was installed between the solvent reservoirs and the injector to trap and delay background PFAS originating from the HPLC system. PFAS were separated by gradient elution using mobile phase 10% (A) and 90% (B) methanol, respectively, with 5 mM ammonium acetate.

QA/QC:

Quality controls included intra and inter batch samples, duplicate/triplicate samples, procedural blanks and instrument blanks in each batch. Identification of PFAS was performed using retention times of the labelled standard. A seven point calibration was carried out to establish relative response factors between natives/^{13}C surrogates and ^{13}C surrogates/^{13}C internal standards, respectively. A calibration standard was injected three times in each batch of samples. Instrument detection limits (LOD) were determined as three times the standard deviation and limit of reporting (LOR) was set to ten times the standard deviation of the concentration of the lowest standard after eight consecutive injections.

Data analysis:

Quantification was carried out over both recorded transitions by isotope dilution for all analytes with exception of native PFPeA, PFHpA, PFBS and PFDS which were quantified against ^{13}C-PFBA, ^{13}C – PFHxA and ^{18}O- PFHxS respectively. Only linear isomers of PFHxS and PFOS were quantified. Complete analytical results for all 13 PFAS are provided in Appendix B.

Data from duplicate/triplicate analysis of sample composites were averaged and the summary of PFOA and PFOS + PFHxS concentrations (maximum, mean and median) for the composite samples from the three site locations are presented in this report. Reported concentrations are in wet weight (ww) for all tissues. All values less than the LOR were assigned a value of ½ reported LOR in the presented results.

2.3. Health Based Guidance Values

Dietary exposure:

The general population is exposed to many sources of PFAS on a daily basis, through air, water, dust, consumer products, occupational exposure and contaminated food [8-10]. Several studies have shown that most people have low level PFAS in their blood serum as a result of this exposure [11-14]. Dietary intake is one of the main pathways of exposure for humans and several studies across different countries have reported elevated PFAS levels in common food groups (e.g. milk, meat, seafood, eggs [8-10]).

In Australia, there is limited data on the levels of PFOA and PFOS from the general food supply, and most data in the literature relate to PFAS levels in food from contaminated sites. The Australian Total Diet study (ATDS), as part of a study to analyse packaging material, tested 50 food types for PFOA and PFOS [15]. PFOS in fish fillets in the general food supply was detected at 1.0 ng g^{-1} and PFOA was not detected (<LOR = 0.4 ng g^{-1}) [15]. A second study carried out by the NSW Food Authority analysed for PFOS, PFOA and PFHxS in 52 imported fish and prawn samples. PFOA and PFHxS were reported at <0.3 ng g^{-1} and PFOS was detected in 2 prawn samples (<LOR – 0.39 ng g^{-1}) [16].

The present study focussed on the dietary exposure via consumption of aquatic foods sourced from three sites, two of which were reported to have elevated levels of PFAS in traditional aquatic food [6]. The daily intake estimates reported in this study are only for a single food group and it is assumed that there is no contribution from other dietary sources (e.g. milk) due to the lack of information available on the variety and amount of different food groups in a day, as well as limited PFAS data from non-contaminated sites.

Determination of health based guidance values:

Health based guidance values are used to assess potential human health risks by indicating the amount of chemical in food or drinking water that a person can consume on a regular basis over a lifetime without significant health risk. The guidance values can be expressed as tolerable monthly intake (TMI), tolerable weekly intake (TWI) or as tolerable daily intake (TDI).

TDIs are usually calculated on the basis of animal studies to which uncertainty factors are applied to account for, for example, inter- and intra- species differences and general uncertainty. The TDI is derived using the most sensitive endpoint in the most relevant study as described below:

$$TDI = \frac{\text{NOAEL or LOAEL}}{\text{UF}}$$

where : NOAEL = no observable adverse effect level
LOAEL = no observable adverse effect level
UF = Uncertainty factor

The TDI is a conservative intake estimate which is based on relevant available toxicological information to which safety (uncertainty) factors are typically applied. Hence, if TDI is exceeded or short periods no negative effect should be expected. Short-term exposure to chemicals exceeding the TDI is therefore usually not a cause of concern, providing the person's average long-term intake does not exceed this limit.

The Australian Government Department of Health engaged the Food Standards Australia New Zealand (FSANZ) to develop final health-based guidance values to replace the interim values adopted by the Environmental Health Standing Committee (enHealth) to assist Australia regulatory authorities to assess the human health risks of site contamination [17]. The new recommended health based guidance values for PFOS and PFOA (Table 2) [7] are lower than the previous guidance values . FSANZ concluded that there was not enough toxicological and epidemiological information to justify establishing a TDI for PFHxS. In practise, PFHxS exposure level should be added to the level of PFOS exposure and this combined level can be compared to the TDI for PFOS.

Table 2 : Tolerable daily intakes (TDI) determined by FSANZ [16]

	PFOS + PFHxS	PFOA
TDI	20 ng/kg bw/day	160 ng/kg bw/day

Calculating Tolerable daily intake:

In this study, the following equation was used to calculate the TDI for each aquatic food species. Due to lack of available data bioavailability or relative absorption of PFAS from food (RAs) was conservatively assumed to be 1.

Equation 1

$$\text{Daily PFAS intake (ng/kg b.w./day)} = \frac{I_S \times C_{PFAS} \times RA_S}{BW}$$

Is - Consumption data (g day^{-1})

C_{PFAS} - Concentration of PFAS (ng g^{-1})

RAs - Relative absorption, assumed to be 1

BW - Body weight in Kg

Two different consumption data sets were used for TDI calculations.

a) As a component of the 2011-13 Australian Health Survey, the 2011-12 National Nutrition and Physical Activity Survey (NNPAS) surveyed 12,153 respondents to determine consumption patterns for the general population [16]. The 90th percentile and mean consumption amounts (g/day) for fish and crustacean muscle are listed in Table 3. Assuming that a single serve equals 150 g, calculated number of serves for both consumption amounts have been listed in Table 3. A body weight of 70 kg was used for adults (male and female) as per EFSA guidelines.

b) The second consumption amounts were based on the number of recommended serves of different aquatic food proposed by FSANZ that can be safely consumed to limit exposure to mercury. This advice is of particular importance for pregnant women and those intending to become pregnant [19]. FSANZ advises only 1 serve (1 serve = 150 grams) of aquatic food for pregnant women if catfish or orange roughy is consumed. If catfish or orange roughy is not consumed, 2-3 serves of any other aquatic fish is recommended. A body weight of 60 Kg was applied for pregnant women in Equation 1.

Table 3 : Consumption amounts based on 2 data sets; NNPAS and 'FSANZ Advice on Fish Consumption'

Species	Tissue	Aquatic food consumption amounts based on Australian consumers (NNPAS)				Recommended aquatic food consumption amounts for pregnant women (FSANZ)	
		P90 (g/day)	Serves*	Mean (g/day)	Serves*	g/day	Serves*
Golden Snapper/barramundi	Muscle	119	5.5	50	2.3	64	3
Catfish	Muscle	119	5.5	50	2.3	21	1
Mudcrab/prawn	Muscle	63	3	21	1	64	3

*Assuming 1 serve = 150 g

Due to less frequent consumption and lack of data on consumption amounts for liver and hepatopancreas these two tissues have been dealt with separately from muscle tissues.

In order to represent the 'worst case' scenario (i.e. if an individual ate aquatic food containing the maximum PFAS concentrations on a long-term and regular basis), the NNPAS consumption amounts for 'high consumer' (90^{th} percentile) and maximum concentrations for PFOA and PFOS + PFHxS in Table 5 for each food type were used to estimate TDI (Section 3.2).

For a more realistic estimation (i.e. if an individual ate aquatic food containing the mean PFAS concentrations on a long-term and regular basis), the NNPAS consumption amounts for an average consumer and mean concentrations for PFOA and PFOS + PFHxS in Table 5 for each food type were used to estimate TDI (Section 3.2). In both scenarios the percentage of TDI was estimated as the percentage contribution of estimated TDI value towards the FSANZ proposed daily PFOA TDI (160 ng/kg bw/day) and PFOS + PFHxS TDI (20 ng/kg bw/day).

In addition to the 'worst case' and more realistic scenarios, TDI and percentage of TDI were determined for pregnant women following the FSANZ advice on fish consumption (in relation to mercury levels) who might be potentially sourcing aquatic food from these three study sites.

Intake from fish and crustaceans were considered independently at first, assuming that exposure from all other food types are at zero while calculating a single food group. Subsequently a more realistic scenario, where an individual consumes different combinations of different foods from all three study sites needed to be considered. Lack of consumption patterns lead to the estimation of daily PFAS intake for different combinations of fish, prawn and mudcrab, using number of serves per week. Amongst several possibilities a selected number of serves and food combinations were considered for mean consumption amount provided by the NNPAS dataset, i.e. 1 and 2 serves per week and FSANZ recommendation for pregnant women, i.e. 1 and 3 serves per week (Table 4). Since FSANZ recommends that no other aquatic food should be consumed by pregnant women if 1 serve of catfish is consumed for the week, PFAS concentrations for catfish were excluded from total PFAS concentrations in order to estimate TDI for aquatic food consumption without catfish. Both consumption datasets were used to estimate TDI at mean and maximum PFAS concentrations. It must be noted that number of serves higher than that reported NNPAS data and FSANZ recommendations have been included to provide more diverse consumption patterns e.g. consuming 9 serves of aquatic food in a week is highly unlikely for an average consumer.

Table 4 : Different combinations and number of serves of aquatic foods selected to calculate TDI and % of TDI

	Aquatic food consumption amounts based on Australian consumers (NNPAS)			Recommended aquatic food consumption amounts for pregnant women (FSANZ)		
	Fish	Mudcrab	Prawn	Fish**	Mudcrab	Prawn
Number of serves*	1	1	1	1	1	1
	2	1	1	3	1	1
	1	2	1	1	3	1
	1	1	2	1	1	3
	1	2	2	1	3	3
	2	2	1	3	3	1
	2	2	2	3	3	3

*Assuming 1 serve = 150 g per week

** Excluding data from catfish muscle

Even accounting for possible combinations of different foods in this study from the same or different locations would not account for other components consumed on a daily basis for e.g. milk, meat and eggs. Therefore, the total daily intake for individuals from these regions will be higher than evaluated in this study.

3. Result and Discussion

3.1. PFAS concentrations in samples

PFOA, PFHxS and PFOS were detected in most samples (91%, 72% and 100% respectively) with PFOS being the most abundant PFAS (Table 5). Due to the small samples size from each site, comparisons between sites are not statistically valid however the data was able to provide preliminary knowledge of potential differences in PFAS levels between the three study sites.

The maximum PFOA levels in mudcrab hepatopancreas (8.6 ng g-1) were higher than for fish muscle (0.063 ng g-1), fish liver (PFOA – 0.59 ng g-1) and crustacean muscle (0.55 ng g-1) (Table 5). The maximum PFOS + PFHxS levels in mudcrab hepatopancreas (180 ng g-1) and fish liver (120 ng g-1) were considerably higher than for fish muscle (7.7 ng g-1) and crustacean muscle (46 ng g-1) (

Table 5).

Mudcrab was the only species analysed from all three sites, and the order of levels observed was Rapid Creek > Ludmilla Creek > Bleesers Creek for both PFOA and PFOS + PFHxS. Mean PFOA concentrations in Ludmilla and Rapid Creeks were up to 4 times higher than levels in Bleesers Creek, and PFOS + PFHxS levels were up to 62 times higher than in Bleesers Creek.

PFOA levels for all species from Bleesers Creek (mudcrab and fish) were similar to PFOA levels in molluscs (i.e. periwinkles and Longbum) from Fright Point, a site with background PFAS levels reported in the previous study [6]; however, PFOS + PFHxS levels in mudcrabs (0.13 ng g^{-1}) were higher in Bleesers Creek than molluscs from Fright point (<LOR), suggesting a PFOS source to Bleesers Creek.

The prawn PFOA levels sampled at Ludmilla Creek were similar to the mudcrab levels from Rapid Creek and double those in the mudcrabs collected from both Ludmilla and Bleesers Creeks. Compared to PFOA, prawn PFOS + PFHxS levels from Ludmilla Creek were relatively higher than the mudcrab levels at all sites, i.e. 4 times higher than Rapid Creek mudcrab levels and 16 and 90 times higher than mudcrabs from Ludmilla and Bleesers, respectively. PFAS levels have previously been reported in mudcrabs and prawns from two estuaries bordering Williamtown RAAF Base (NSW), a known PFAS contaminated site [18]. The highest mudcrab PFAS levels measured in the present study, i.e. from Rapid Creek (PFOA – 0.31 ng g^{-1}, PFOS + PFHxS – 8.0 ng g^{-1}), were higher than the maximum levels in mudcrabs from Williamtown (PFOA – <0.3 ng g^{-1} , PFOS + PFHxS 2.1 – 4.2 ng g^{-1}). PFAS prawn levels from Ludmilla Creek (PFOA – 0.24 ng g^{-1}, PFOS + PFHxS – 32 ng g^{-1}) were in the range detected in prawns from Williamtown (PFOA – <0.3 ng g^{-1}, PFOS + PFHxS 17 – 42 ng g^{-1}) [18].

Fish samples were collected from two sites, Bleesers Creek and Ludmilla Creek. In fish muscle, no difference was observed between mean PFOA levels between the sites. Consistent with the mudcrab levels from these two sites, PFOS + PFHxS levels were lowest in fish from Bleesers Creek (0.16 – 0.37 ng g^{-1}) and highest in fish from Ludmilla Creek (5.6 ng g^{-1}). In comparison, PFOS + PFHxS level in fish from Ludmilla Creek (5.6 ng g^{-1}) was similar to levels in fish sourced from Williamtown (3.4 – 9.0 ng g^{-1}) [18].

Table 5 : Summary of PFOA and PFOS + PFHxS concentrations (ng g^{-1} ww) from three study sites

Species	Tissue	Location	n*	PFOA (ng g^{-1} ww)			PFOS + PFHXs (ng g^{-1}ww)		
				Max	Mean	Median	Max	Mean	Median
Golden Snapper	Muscle	Bleesers Creek	2	0.063	0.048	0.048	0.20	0.16	0.16
Catfish	Liver	Bleesers Creek	2	0.21	0.19	0.19	12	11	11
Catfish	Muscle	Bleesers Creek	2	0.052	0.037	0.037	0.42	0.37	0.37
Mudcrab	Muscle	Bleesers Creek	1	0.080	0.080	0.080	0.13	0.13	0.13
Mudcrab	Muscle	Rapid Creek	3	0.54	0.31	0.20	12	8.0	9.2
Mudcrab	Hepatopancreas	Rapid/Ludmilla	2	8.6	5.1	5.1	180	110	110
Mudcrab	Muscle	Ludmilla Creek	2	0.13	0.11	0.11	3.0	2.0	2.0
Barramundi	Liver	Ludmilla Creek	2	0.59	0.44	0.44	120	100	100
Barramundi	Muscle	Ludmilla Creek	2	0.056	0.047	0.047	7.7	5.6	5.6
Prawn	Muscle	Ludmilla Creek	5	0.55	0.24	0.17	46	32	40

*** -number of composites**

Due to 1 or 2 composites from a site, values for mean and median are identical

3.2. Daily PFAS intake for aquatic foods

Daily PFOA intake for Independently estimated aquatic foods

a) Adult consumers

Estimated maximum daily intake for PFOA from fish and crustaceans were considered independently using NNPAS consumption amounts (g/day) and are presented in

Table 6.

Assuming the maximum PFOA concentrations reported in this study and the 'high consumer' amount of aquatic food consumed on a regular basis as reported by NNPAS, the 'worst case' PFOA intake from the fish and crustaceans in this study would contribute at most 0.31% of the TDI.

For example, for an individual consuming mudcrab muscle from Rapid Creek at the maximum PFOA concentration detected in this study (0.54 ng g-1) and assuming a 70 kg body weight, the PFOA daily intake would be 0.49 ng/kg bw/day, i.e. 0.30% of the TDI of 160 ng/kg bw/day. This individual would have to consume approximately 21 kg of mudcrab (with PFOA concentration - 0.54 ng g-1) daily before reaching the TDI.

b) Pregnant women

As per the FSANZ advice on fish consumption (in relation to mercury levels) [19], pregnant women are recommended only 1 serving of catfish (150 g) per week which calculates to approximately 21 g/day. At the maximum PFOA concentration found in this study (0.052 ng g-1) and assuming a body weight of 60 kg, the PFOA daily intake for a pregnant woman consuming the recommended amount of catfish would be 0.018 ng/kg bw/day, i.e. 0.01% of the TDI of 160 ng/kg bw/day. This individual would have to consume 184 kg of catfish (with PFOA concentration - 0.052 ng g-1) daily before reaching the TDI.

Table 6: Estimated TDI (ng/kg bw/day) and % of TDI at maximum PFOA levels for 2 consumption amounts and 2 different body weights from 3 study sites

Species	Tissue	Location	Using NNPAS data Adult (70 kg)				Using FSANZ data Pregnant women (60 Kg)	
			High consumer		Average consumer			
			ng/kg bw/day	TDI %	ng/kg bw/day	TDI %	ng/kg bw/day	TDI %
Golden Snapper	Muscle	Bleesers Creek	0.11	0.07%	0.051	0.03%	0.067	0.04%
Catfish	Muscle	Bleesers Creek	0.088	0.06%	0.013	0.01%	0.018	0.01%
Mudcrab	Muscle	Bleesers Creek	0.072	0.05%	0.085	0.05%	0.085	0.05%
Mudcrab	Muscle	Rapid Creek	0.49	0.30%	0.33	0.21%	0.58	0.36%
Mudcrab	Muscle	Ludmilla Creek	0.12	0.07%	0.12	0.07%	0.14	0.09%
Barramundi	Muscle	Ludmilla Creek	0.095	0.06%	0.050	0.03%	0.060	0.04%
Prawn	Muscle	Ludmilla Creek	0.50	0.31%	0.26	0.16%	0.59	0.37%

Assessing long term exposure from three sites and three aquatic food types

Table 7 presents the estimated daily PFOA intake for mean consumption amounts (reported by NNPAS) at mean and maximum PFOA concentrations for different number of serves and different food groups. Assuming that an individual consumes aquatic food from all three study sites over a lifetime, the highest estimated percentage TDI in this study for selected combinations were, 0.16% calculated at mean concentration and 0.44% at maximum concentration. Using FSANZ data for pregnant women (

Table 8), the highest estimated percentage TDI in this study for the selected combinations were, 0.25% calculated at mean concentration and 0.66% at maximum concentration.

Table 7 : Estimated TDI (ng/kg bw/day) and % of TDI at mean and maximum PFOA levels for different combinations of aquatic foods for mean consumption amounts (NNPAS)

	Aquatic Food type			Mean Conc		Max Conc	
	Fish	Mudcrab	Prawn	TDI	TDI %	TDI	TDI %
Number of serves*	1	1	1	0.13	0.08%	0.35	0.22%
	2	1	1	0.14	0.09%	0.37	0.23%
	1	2	1	0.17	0.11%	0.52	0.32%
	1	1	2	0.20	0.13%	0.52	0.33%
	1	2	2	0.25	0.15%	0.69	0.43%
	2	2	1	0.19	0.12%	0.54	0.34%
	2	2	2	0.26	0.16%	0.71	0.44%

*Assuming 1 serve = 150 g per week

Table 8 : Estimated TDI (ng/kg bw/day) and % of TDI at maximum PFOA levels for different combinations of aquatic foods for pregnant women (FSANZ)

Number of serves*	Aquatic Food type			Mean Conc		Max Conc	
	Fish	Mudcrab	Prawn	TDI	TDI %	TDI	TDI %
	1	1	1	0.13	0.08%	0.35	0.22%
	3	1	1	0.17	0.11%	0.39	0.24%
	1	3	1	0.22	0.14%	0.68	0.43%
	1	1	3	0.28	0.17%	0.69	0.43%
	1	3	3	0.37	0.23%	1.02	0.64%
	3	3	1	0.25	0.15%	0.72	0.45%
	3	3	3	0.39	0.25%	1.06	0.66%

*Assuming 1 serve = 150 g per week

Daily PFOS + PFHxS intake for Independently estimated aquatic foods

a) Adult consumers

The TDI value for PFOS + PFHxS is 8-fold lower than PFOA and consequently the estimated amount of each site's food that could be consumed before the TDI was reached was much lower than for PFOA. Estimated maximum daily intake for PFOS + PFHxS from fish and crustaceans were considered independently for two different adult body weights using NNPAS consumption amounts (g/day) and are presented in Table 9.

Assuming the maximum PFOS + PFHxS concentrations reported in this study and the 'high consumer' amount of aquatic food consumed on a regular basis as reported by NNPAS, the 'worst case' PFOS + PFHxS intake estimated from the fish and crustaceans in this study exceed the TDI for prawn muscle (<200%). For prawns, if an individual (70 kg bw) were to consume 30 g of prawns from Ludmilla Creek daily, or approximately 200 g a week, at the maximum detected PFOS + PFHxS concentration (46 ng g^{-1}) in this study, they would reach the TDI threshold of 20 ng/kg bw/day. Daily intake would be exceeded for an average consumer at mean PFOS + PFHxS concentration detected in this study.

However, it is highly unlikely that an individual would source food from the same location throughout their lifetime and consistently consume prawns with PFOS + PFHxS detected at maximum or mean levels reported in this study.

For mudcrabs, an individual consuming 32g/day from Rapid Creek at the maximum PFOS + PFHxS concentration detected in this study (12 ng g-1) and assuming a 70 kg body weight, the PFOS + PFHxS daily intake would be 11 ng/kg bw/day, i.e. 54% of the TDI of 20 ng/kg bw/day. In comparison, if an individual were to consume mudcrab from Ludmilla Creek at the maximum PFOS + PFHxS concentration detected in this study (3.0 ng g-1) or Bleesers Creek (0.13 ng g-1) the respective % of TDI was estimated at 14% and 0.59%.

The highest PFOS + PFHxS concentrations detected in fish were from barramundi found in Ludmilla Creek (7.7 ng g^{-1}). Assuming the maximum PFOS + PFHxS concentration, the daily intake for an individual (70 kg) would be 13 ng/kg bw/day, i.e. 65 % of the TDI of 20 ng/kg bw/day.

b) Pregnant women

At the maximum PFOS + PFHxS concentration found in this study (0.42 ng g^{-1}) and assuming a body weight of 60 kg, the PFOS + PFHxS daily intake for a pregnant woman consuming the recommended amount of catfish would be 0.15 ng/kg bw/day, i.e. 0.7% of the TDI of 20 ng/kg bw/day. This individual would have to consume 2.8 kg of catfish muscle (PFOS + PFHxS concentration - 0.42 ng g^{-1}) daily before reaching the TDI threshold. Applying higher maximum concentration detected in barramundi muscle (PFOS + PFHxS concentration – 7.7 ng g^{-1}), the PFOS + PFHxS daily intake for a pregnant woman consuming the recommended amount of fish would be 8.2 ng/kg bw/day, i.e. 41% of the TDI of 20 ng/kg bw/day. The individual would have to consume 155 g of barramundi muscle (PFOS + PFHxS concentration – 7.7 ng g-1) daily before reaching the TDI threshold. PFOS + PFHxS intake estimated from the fish and crustaceans in this study exceeded the TDI for prawn muscle (>200%), the values were similar to those calculated from NNPAS data.

Table 9 : Estimated TDI (ng/kg bw/day) and % of TDI at maximum PFOS + PFHxS levels for 2 consumption amounts and 2 different body weights from 3 study sites

Species	Tissue	Location	Using NNPAS data Adult (70 kg)				Using FSANZ data Pregnant women (60 Kg)	
			High consumer		Average consumer			
			ng/kg bw/day	TDI %	ng/kg bw/day	TDI %	ng/kg bw/day	TDI %
Golden Snapper	Muscle	Bleesers Creek	0.34	1.7%	0.17	0.9%	0.21	1.1%
Catfish	Muscle	Bleesers Creek	0.71	3.6%	0.13	0.6%	0.15	0.7%
Mudcrab	Muscle	Bleesers Creek	0.12	0.6%	0.14	0.7%	0.14	0.7%
Mudcrab	Muscle	Rapid Creek	11	54%	8.5	43%	13	8.0%
Mudcrab	Muscle	Ludmilla Creek	2.7	14%	2.1	11%	3.2	16%
Barramundi	Muscle	Ludmilla Creek	13	65%	6.0	30%	8.2	41%
Prawn	Muscle	Ludmilla Creek	41	210%	34	170%	49	250%

Daily PFOS + PFHxS intake for combined different aquatic foods from the same site

Table 10 presents the estimated daily PFOS + PFHxS intake for mean consumption amounts reported by NNPAS at mean and maximum PFOS + PFHxS concentrations for different number of serves and different food groups. Assuming that an individual consumes aquatic food from all three study sites over a lifetime, the highest estimated percentage TDI for selected combinations were, 113% calculated at mean concentration and 200% at maximum concentration in this study. The TDI threshold was reached or exceeded with the inclusion of prawns (maximum or mean concentrations from this study) in an individual's diet. Using FSANZ data for pregnant women (

Table 11), the highest estimated percentage TDI for the selected combinations were, 170% calculated at mean concentration and 302% at maximum concentration in this study. As previously mentioned it is highly unlikely that an individual would source food from the same location throughout their lifetime and consistently consume prawns with PFOS + PFHxS detected at maximum or mean levels reported in this study.

Table 10 : Estimated TDI (ng/kg bw/day) and % of TDI at mean and maximum PFOS + PFHxS levels for different combinations of aquatic foods for mean consumption amounts (NNPAS)

Number of serves*	Aquatic Food type			Mean Conc		Max Conc	
	Fish	Mudcrab	Prawn	TDI	TDI %	TDI	TDI %
	1	1	1	11	56%	20	101%
	2	1	1	12	60%	22	112%
	1	2	1	12	61%	24	119%
	1	1	2	21	105%	34	171%
	1	2	2	22	110%	38	190%
	2	2	1	13	64%	26	130%
	2	2	2	23	113%	40	200%

*Assuming 1 serve = 150 g per week

Table 11 : Estimated TDI (ng/kg bw/day) and % of TDI at maximum PFOS + PFHxS levels for different combinations of aquatic foods for pregnant women (FSANZ)

Number of serves*	Aquatic Food type			Mean Conc		Max Conc	
	Fish	Mudcrab	Prawn	TDI	TDI %	TDI	TDI %
	1	1	1	12	58%	20	101%
	3	1	1	13	67%	25	120%
	1	3	1	13	65%	27	140%
	1	1	3	31	150%	48	240%
	1	3	3	33	160%	56	280%
	3	3	1	14	71%	32	160%
	3	3	3	34	170%	60	302%

*Assuming 1 serve = 150 g per week

Dietary assessment for hepatopancreas and fish liver consumed as a 'Delicacy'

Though hepatopancreas and liver are not regularly consumed by the general public, they are consumed as a delicacy by some cultural groups. It was not possible to estimate the TDI or % of TDI for hepatopancreas and liver due to lack of data on consumption amounts. Instead consumption amounts were calculated for liver/hepatopancreas (independently) to reach TDI PFAS threshold of (PFOA - 160 ng/kg bw/day and PFOS + PFHxS - 20 ng/kg bw/day) by applying maximum and mean PFAS concentrations from this study. Due to the lower PFOA concentrations in this study and higher TDI threshold, consumption amounts were many fold higher than reported NNPAS 'high consumer' values. However, higher PFOS + PFHxS levels in this study and lower TDI threshold resulted in reasonable consumption amounts for liver and hepatopancreas and are presented in Table 12.

Table 12 : Consumption amount estimated to reach TDI (20 ng/kg bw/day) for 2 body weights using maximum and mean PFOS + PFHxS concentrations

Species	Tissue	Location	Consumption amount (g/day) to reach TDI (20 ng/kg bw/day) for b.w 70 kg		Consumption amount (g/day) to reach TDI (20 ng/kg bw/day) for b.w 60 kg	
			Maximum concentration	Mean concentration	Maximum concentration	Mean concentration
Catfish	Liver	Bleesers Creek	120	130	100	109
Mudcrab	Hepatopancreas	Rapid/Ludmilla	7.8	13	6.7	11
Barramundi	Liver	Ludmilla Creek	12	14	10	12

Though it is likely for a consumer to reach or exceed the TDI threshold for PFOS + PFHxS, by consuming 6.7 g/day or approximately 47 g per week of mudcrab hepatopancreas, it is highly unlikely that an individual (60 kg) would consume 6.7 g/day of hepatopancreas daily at the maximum PFOS + PFHxS concentration reported in this study over a lifetime.

3.3. Assessment of overall risk from consuming aquatic foods containing PFAS from Ludmilla, Rapid and Bleesers Creeks

Based on the calculations presented in Section 3.2, a few combinations of food types and also individual foods from certain study sites resulted in a daily PFAS intake exceeding the proposed FSANZ values. In addition to no adverse health effects caused from occasionally exceeding TDI thresholds it may be unexpected for an individual to source food from the same locality on a consistent basis, making the chance of long-term exceedance of the TDI unlikely. For e.g. in one scenario, to reach the TDI, a person (70 kg bw) would have to consume 30 g/day of prawns from Ludmilla Creek, containing the maximum PFOS + PFHxS levels reported in this study, each week consistently over their lifetime.

As mentioned in section 3.1.2 tolerable daily intake values are a conservative estimate for a lifetime of exposure, and exceedance over the threshold for short periods is not generally associated with adverse health effects [20]. It is also important to note that the TDIs for PFOA and PFOA + PFHxS are precautionary measures given that there is, at present, no consistent evidence that exposure to PFAS causes adverse health effects. The recommended TDI values allow for a large margin of safety to protect public health. For example, the PFOS + PFHxS TDI of 20 ng/kg bw/day is based on decreased weight gain in a toxicity study of rats [21] together with pharmacokinetic modelling to calculate the equivalent human 'no observed adverse effect level' dose. An uncertainty (safety) factor of 30 was applied, which comprised a factor of 3 to account for interspecies differences in toxicodynamics (i.e. rats versus humans) and a factor of 10 for intra-species differences within the human population (i.e. between different people) [21]. As indicated by Bartholomaeus [20], health based guidance values such as TDIs "are not…bright lines between safety and risk, but rather represent the limit of confidence in the safe intake level". When an intake exceeds the TDI, it means that the confidence in there being no risk (which includes a safety factor of 30 in the case of PFOS + PFHxS) is being eroded. The likelihood that some risk will be associated with that exposure increases with length of regular exposure above the TDI [20].

It should be noted that in the presented scenarios we have assumed that exposure from all other foods in a person's diet were at zero while calculating a single food group. This was also true while estimating daily PFAS intake using different combinations of food. This may not be a true representation of a person's diet. Other commodities such as milk, eggs, meat, vegetables and fruits have shown to have detectable PFAS concentrations [8-10]. This means that the daily PFAS intake, as estimated in Section 3.2, may be underestimated.

4. Conclusion

4.1. PFAS levels in aquatic samples

All species of fish and crustaceans analysed were detected at higher mean PFAS levels in Rapid or Ludmilla Creek in comparison to Bleesers Creek. However, mudcrab was the only species analysed from all three sites. PFOA levels detected in mudcrabs from Bleesers Creek were similar to levels in molluscs from Fright Point site, a reference site from the previous study in the region. However PFOS + PFHxS levels were higher in Bleesers Creek than in Fright Point suggesting a PFOS source around Bleesers Creek.

The highest mean mudcrab PFOA and PFOS + PFHxS levels from Rapid Creek were higher than mudcrab levels reported from two estuaries bordering Williamtown RAAF Base, a known contaminated site.

Prawn PFOA levels from Ludmilla Creek were similar to mudcrab levels from Rapid Creek and 2 times higher than mudcrab levels from Bleesers and Ludmilla Creeks. Compared to PFOA, prawn PFOS + PFHxS levels from Ludmilla Creek were relatively higher than the mudcrab levels from Ludmilla and Bleesers Creek and were in the concentration range detected in prawns from Williamtown.

Fish samples were collected from two sites, Bleesers Creek and Ludmilla Creek, although due to opportunistic sampling, different species were caught at each site. No difference was observed between mean PFOA levels between the two sites but the higher PFOS + PFHxS levels detected in Ludmilla Creek, compared to Bleesers Creek, were consistent with fish data from Williamtown. Further sampling would be necessary to confirm that PFAS levels in fish from Ludmilla and Rapid Creeks are consistently higher than Bleesers Creek.

Overall, this initial study suggests that Rapid and Ludmilla Creeks are more impacted by PFAS contamination than Bleesers Creek. Levels from mudcrabs, prawns and fish are mostly consistent or slightly higher than those analysed from Williamtown RAAF Base, a known PFAS-contaminated site.

4.2. Assessment of dietary exposure

Based on the maximum PFOA and PFOS + PFHxS levels measured in this study, TDI was estimated using two separate consumption data sets of fish and crustaceans for two different body weights. It should be noted that this report assessed consumption of aquatic food types sampled in this study as a single dietary source and, due to the lack of reported PFAS levels in other regularly consumed foods such as milk, eggs and meat, an overall dietary daily intake of PFAS has not be estimated.

Assuming zero exposure from other food types, TDI estimates using both NNPAS and FSANZ consumption amounts were lower than the TDI threshold values (PFOA – 160 ng/kg bw/day and PFOS + PHFxS – 20 ng/kg bw/day) for most species. This held true for:

I. PFOA for all food species and all creeks
II. PFOS + PFHxS for all aquatic food species from Bleesers Creek
III. PFOS + PFHxS for mudcrab muscle from all creeks.
IV. PFOS + PFHxS for barramundi muscle from Ludmilla Creek

In general, at the highest expected consumption levels based in the NNPAS survey of the Australian population and for the most contaminated species, consuming these aquatic foods (I to IV) on a daily basis would contribute <65% of the TDI (for a 70 kg adult, the highest percentage would be 65%, relating to consumption of barramundi muscle from Ludmilla Creek, followed by 41% relating to consumption of mudcrab muscle from Rapid Creek).

There were exceptions where consumption of aquatic foods from these creeks may lead to a daily PFAS intake exceeding the TDI. These were:

V. PFOS + PFHxS for prawn muscle from Ludmilla Creek
VI. PFOS + PFHxS for mudcrab hepatopancreas from Rapid/Ludmilla Creek, catfish liver from Bleesers Creek and barramundi liver from Ludmilla Creek

In addition to estimating TDI independently for different food types, TDI estimations were considered in a few selected combinations of food types from the three study sites. In general, none of the combinations exceeded the TDI threshold for PFOA. However, certain combinations execeeded the TDI threshold for PFOs + PFHxS, for e.g. estimated TDI exceeded the PFOS + PFHxS TDI threshold when a single serve of prawn from Ludmilla Creek was included along with fish and mudcrab per week.

For case V and VI, it is important to note that the risk of adverse health effects is only expected when these aquatic foods are eaten on a regular, weekly basis over an individual's lifetime. Lack of consumption amounts for hepatopancreas and liver did not allow for reliable TDI estimation. Furthermore, exceedances for e.g. in TDI for prawn consumption is also only expected when consistently eating amounts that are considered 'high' compared to the general Australian population and assuming that every prawn caught and consumed has the maximum PFOS + PFHxs concentration reported in this study. For all three species, it is not expected that people would source food from the same locality on a consistent basis over their lifetimes, thus a long-term exceedance of the TDI is considered unlikely.

5. References

1. Giesy, J.P. and K. Kannan, Global distribution of perfluorooctane sulfonate in wildlife. Environmental Science and Technology, 2001. 35(7): p. 1339-1342.
2. Buck, R., Murphy, P and Pabon, M, Chemistry, Properties, and Uses of Commercial Fluorinated Surfactants, The Handbook of Environmental Chemistry in Polyfluorinated Chemicals and Transformation Products, Springer, Editor. 2011. p. 1-24.
3. K. Prevedouros, I.T.C., R.C. Buck, S.H. Korzeniowski, Sources, Fate and Transport of Perfluorocarboxylates. Environmental Science & Technology, 2006. 40.
4. Vierke, L., A. Moller, and S. Klitzke, Transport of perfluoroalkyl acids in a water-saturated sediment column investigated under near-natural conditions. Environmental Pollution, 2014. 186: p. 7-13.
5. Houtz, E.F., et al., Persistence of Perfluoroalkyl Acid Precursors in AFFF-Impacted Groundwater and Soil. Environmental Science & Technology, 2013. 47(15): p. 8187-8195.
6. Niels C. Munksgaard, D.L., Karen S. Gibb, Donna Jackson, Sharon Grant, Jennifer Braeunig and Jochen F. Mueller Per- and Polyfluoroalkyl Substances (PFAS) testing in sediment and aquatic foods from Darwin Harbour. 2017.
7. Australian Government, Department of Health. Health based Guidance Values for PFAS for use in site investigations in Australia. 2017; Available from: https://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE086D480353CA2580C900817CDC/$File/fs-Health-Based-Guidance-Values.pdf.
8. Ericson, I., et al., Human exposure to perfluorinated chemicals through the diet: Intake of perfluorinated compounds in foods from the Catalan (Spain) market. Journal of Agricultural and Food Chemistry, 2008. 56(5): p. 1787-1794.
9. Fromme, H., et al., Perfluorinated compounds - Exposure assessment for the general population in western countries. International Journal of Hygiene and Environmental Health, 2009. 212(3): p. 239-270.
10. Haug, L.S., et al., Characterisation of human exposure pathways to perfluorinated compounds - Comparing exposure estimates with biomarkers of exposure. Environment International, 2011. 37(4): p. 687-693.
11. Calafat, A.M., et al., Polyfluoroalkyl chemicals in the US population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environmental Health Perspectives, 2007. 115(11): p. 1596-1602.
12. Kato, K., et al., Trends in Exposure to Polyfluoroalkyl Chemicals in the US Population: 1999-2008. Environmental Science & Technology, 2011. 45(19): p. 8037-8045.
13. Schroter-Kermani, C., et al., Retrospective monitoring of perfluorocarboxylates and perfluorosulfonates in human plasma archived by the German Environmental Specimen Bank. International Journal of Hygiene and Environmental Health, 2013. 216(6): p. 633-640.
14. Toms, L.M.L., et al., Decline in perfluorooctane sulfonate and perfluorooctanoate serum concentrations in an Australian population from 2002 to 2011. Environment International, 2014. 71: p. 74-80.
15. Food Standards Australia New Zealand. 24th Australian Total Diet Study, FSANZ Australia, Canberra, Australia. 2016.
16. Australian Government, D.o.H. Supporting document 2: Assessment of potential dietary exposure to perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonate (PFHxS) occurring in foods sampled from contaminated sites. 2017; Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE086D480353CA2580C900817CDC/$File/Dietary-Exposure-Assesment.pdf.

17. Environmental Health Standing Commitee (enHealth). Interim national guidance on human health reference values for per- and poly-fluoroalkyl substances for use in site investigations in Australia. . 2016; Available from: http://www.health.nsw.gov.au/environment/factsheets/Documents/pfas-interim-health-values-ahppc.pdf.
18. Taylor, M.D. and D.D. Johnson, Preliminary investigation of perfluoroalkyl substances in exploited fishes of two contaminated estuaries. Marine Pollution Bulletin, 2016. 111(1-2): p. 509-513.
19. Food Standards Australia New Zealand - Mercury in Fish. Available from: http://www.foodstandards.gov.au/consumer/chemicals/mercury/Pages/default.aspx.
20. Bartholomaeus, A., Procedural Review of Health Reference Values Established by enHealth for PFAS 2016.
21. FSANZ - Hazard assessment report – Perfluorooctane sulfonate (PFOS), Perfluorooctanoic Acid (PFOA), Perfluorohexane sulfonate (PFHxS). Report to Department of Health. 2017; Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE086D480353CA2580C900817CDC/$File/Summary-Hazard-Assessment-Report-PFOS-PFOA-PFHxS.pdf.

Appendix A

Table A1 : Muscle sample list and corresponding composites

Fish ID	Date	Species	Common name	Location	Total length (mm)	Composite ID
PFAS001	4/11/2016	*L. johnii*	Golden Snapper	Bleesers Creek	200	SNAPM_BC1
PFAS002	4/11/2016	*L. johnii*	Golden Snapper	Bleesers Creek	240	
PFAS003	4/11/2016	*L. johnii*	Golden Snapper	Bleesers Creek	250	SNAPM_BC2
PFAS004	4/11/2016	*L. johnii*	Golden Snapper	Bleesers Creek	365	
PFAS020	4/11/2016	*L. johnii*	Golden Snapper	Bleesers Creek	340	
PFAS012	4/11/2016	*N. graeffei*	Blue Catfish	Bleesers Creek	400	CATM_BC1
PFAS017	4/11/2016	*N. graeffei*	Blue Catfish	Bleesers Creek	465	
PFAS019	4/11/2016	*N. graeffei*	Blue Catfish	Bleesers Creek	385	
PFAS006	4/11/2016	*N. graeffei*	Blue Catfish	Bleesers Creek	445	CATM_BC2
PFAS013	4/11/2016	*N. graeffei*	Blue Catfish	Bleesers Creek	485	
PFAS018	4/11/2016	*N. graeffei*	Blue Catfish	Bleesers Creek	410	
PFAS021	4/11/2016	*S. serrata*	Mudcrab	Bleesers Creek	160	MUDM_BC1
PFAS022	4/11/2016	*S. serrata*	Mudcrab	Bleesers Creek	168	
PFAS023	4/11/2016	*S. serrata*	Mudcrab	Bleesers Creek	172	
PFAS045	17/11/2016	*S. serrata*	Mudcrab	Rapid Creek	163	MUDM_RC1
PFAS048	17/11/2016	*S. serrata*	Mudcrab	Rapid Creek	155	
PFAS052	17/11/2016	*S. serrata*	Mudcrab	Rapid Creek	145	
PFAS046	17/11/2016	*S. serrata*	Mudcrab	Rapid Creek	168	MUDM_RC2
PFAS049	17/11/2016	*S. serrata*	Mudcrab	Rapid Creek	130	
PFAS051	17/11/2016	*S. serrata*	Mudcrab	Rapid Creek	127	
PFAS047	17/11/2016	*S. serrata*	Mudcrab	Rapid Creek	132	MUDM_RC3
PFAS050	17/11/2016	*S. serrata*	Mudcrab	Rapid Creek	135	
PFAS053	17/11/2016	*S. serrata*	Mudcrab	Rapid Creek	165	
PFAS040	17/11/2016	*S. serrata*	Mudcrab	Ludmilla Creek	158	MUDM_LD1
PFAS042	17/11/2016	*S. serrata*	Mudcrab	Ludmilla Creek	145	
PFAS043	17/11/2016	*S. serrata*	Mudcrab	Ludmilla Creek	132	
PFAS041	17/11/2016	*S. serrata*	Mudcrab	Ludmilla Creek	105	MUDM_LD2
PFAS044	17/11/2016	*S. serrata*	Mudcrab	Ludmilla Creek	156	
PFAS074	21/11/2016	*L. calcarifer*	Barramundi	Ludmilla Creek	103	BARRAM_LD1
PFAS077	21/11/2016	*L. calcarifer*	Barramundi	Ludmilla Creek	48	
PFAS078	21/11/2016	*L. calcarifer*	Barramundi	Ludmilla Creek	61	
PFAS079	21/11/2016	*L. calcarifer*	Barramundi	Ludmilla Creek	47	
PFAS075	21/11/2016	*L. calcarifer*	Barramundi	Ludmilla Creek	51	BARRAM_LD2
PFAS076	21/11/2016	*L. calcarifer*	Barramundi	Ludmilla Creek	47	
PFAS080	21/11/2016	*L. calcarifer*	Barramundi	Ludmilla Creek	53	
PFAS081	21/11/2016	*L. calcarifer*	Barramundi	Ludmilla Creek	46	
PFAS083	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	52	PRAWM_LD1
PFAS084	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	60	
PFAS085	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	65	
PFAS086	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	76	
PFAS087	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	77	
PFAS088	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	85	PRAWM_LD2
PFAS089	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	86	
PFAS090	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	86	
PFAS091	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	90	PRAWM_LD3
PFAS092	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	89	
PFAS093	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	98	
PFAS094	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	99	PRAWM_LD4
PFAS095	31/03/2017	Metapenaeus sp.	Prawn	Ludmilla Creek	104	
PFAS096	31/03/2017	*P. monodon*	Prawn	Ludmilla Creek	135	PRAWM_LD5

Table A2 : Liver and hepatopancreas sample list and corresponding composites

Fish ID	Date	Species	Common name	Tissue	Location	TL (mm)	Composite ID
PFAS019	4/11/2016	N. *graeffei*	Blue Catfish	Liver	Bleesers Creek	385	CATL_BC1
PFAS017	4/11/2016	N. *graeffei*	Blue Catfish	Liver	Bleesers Creek	465	
PFAS012	4/11/2016	N. *graeffei*	Blue Catfish	Liver	bleesers Creek	400	
PFAS006	4/11/2016	N. *graeffei*	Blue Catfish	Liver	bleesers Creek	445	CATL_BC2
PFAS013	4/11/2016	N. *graeffei*	Blue Catfish	Liver	bleesers Creek	485	
PFAS018	4/11/2016	N. *graeffei*	Blue Catfish	Liver	bleesers Creek	410	
PFAS079	21/11/2016	L. *calcarifer*	Barramundi	Liver	Ludmilla Creek	47	BARRAL_LD1
PFAS074	21/11/2016	L. *calcarifer*	Barramundi	Liver	Ludmilla Creek	103	
PFAS081	21/11/2016	L. *calcarifer*	Barramundi	Liver	Ludmilla Creek	46	
PFAS075	21/11/2016	L. *calcarifer*	Barramundi	Liver	Ludmilla Creek	51	
PFAS076	21/11/2016	L. *calcarifer*	Barramundi	Liver	Ludmilla Creek	47	BARRAL_LD2
PFAS077	21/11/2016	L. *calcarifer*	Barramundi	Liver	Ludmilla Creek	48	
PFAS078	21/11/2016	L. *calcarifer*	Barramundi	Liver	Ludmilla Creek	61	
PFAS080	21/11/2016	L. *calcarifer*	Barramundi	Liver	Ludmilla Creek	53	
PFAS060	17/11/2016	S. *serrata*	Mudcrab	Hepatopancreas	Rapid/Ludmilla	N/A	MUDH_R/LD1
PFAS061	17/11/2016	S. *serrata*	Mudcrab	Hepatopancreas	Rapid/Ludmilla	N/A	
PFAS062	17/11/2016	S. *serrata*	Mudcrab	Hepatopancreas	Rapid/Ludmilla	N/A	
PFAS068	17/11/2016	S. *serrata*	Mudcrab	Hepatopancreas	Rapid/Ludmilla	N/A	
PFAS058	17/11/2016	S. *serrata*	Mudcrab	Hepatopancreas	Rapid/Ludmilla	N/A	MUDH_R/LD2
PFAS071	17/11/2016	S. *serrata*	Mudcrab	Hepatopancreas	Rapid/Ludmilla	N/A	
PFAS069	17/11/2016	S. *serrata*	Mudcrab	Hepatopancreas	Rapid/Ludmilla	N/A	

Appendix B

Table B1 : Analytical PFAS results for samples from Bleesers Creek (BC); M – muscle, L – liver; CAT – Catfish, MUD –Mudcrab, SNAP – Golden snapper; rep – replicate; BOLD – recovery <15%

Composite ID	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFBS	PFHxS	PFOS	PFUnDA	PFDODA	PFDS	PFHxS/PFOS
ng/g - LOR	0.04	0.05	0.03	0.01	0.02	0.02	0.04	0.04	0.02	0.04	0.15	0.08	0.02	
CATM_BC1	ND	ND	ND	ND	0.14	ND	ND	ND	ND	0.52	0.06	ND	ND	0.52
CATM_BC1_rep	<0.04	ND	ND	ND	<0.02	ND	ND	ND	ND	0.35	ND	ND	ND	0.35
CATM_BC1_rep	ND	ND	ND	ND	<0.02	0.02	ND	ND	0.02	0.34	ND	ND	ND	0.36
CATL_BC1	ND	ND	ND	ND	0.11	0.33	0.22	ND	ND	10.23	**0.24**	ND	ND	10.23
CATL_BC1_rep	ND	ND	ND	ND	0.14	0.44	ND	ND	ND	8.80	**0.37**	ND	ND	8.80
CATL_BC1_rep	ND	ND	ND	ND	0.39	0.45	0.21	ND	0.24	8.98	**0.48**	ND	ND	9.22
CATM_BC2	ND	ND	ND	ND	ND	0.02	ND	ND	0.03	0.29	<0.15	ND	ND	0.31
CATM_BC2_rep	ND	ND	ND	ND	0.03	0.03	ND	ND	0.02	0.32	<0.15	ND	ND	0.34
CATM_BC2_rep	ND	ND	ND	ND	0.03	ND	ND	ND	ND	0.29	<0.15	ND	ND	0.29
CATL_BC2	ND	ND	ND	ND	0.16	0.59	**0.29**	ND	ND	12.11	**0.61**	ND	ND	12.11
CATL_BC2_rep	ND	ND	ND	ND	0.24	0.59	0.28	ND	0.33	10.88	**0.82**	**0.28**	ND	11.21
CATL_BC2_rep	ND	ND	ND	ND	0.12	0.68	0.20	ND	**0.75**	11.72	**0.52**	**1.05**	ND	12.47
MUDM_BC1	ND	0.02	0.04	0.00	0.09	0.06	0.04	<0.04	<0.02	0.13	0.11	0.12	<0.02	0.13
MUDM_BC1_rep	0.18	0.02	0.03	0.01	0.07	0.04	**0.04**	<0.04	<0.02	0.11	**0.11**	**0.22**	<0.02	0.11
SNAPM_BC1	0.05	<0.05	ND	ND	0.04	ND	ND	ND	<0.02	0.12	ND	0.01	ND	0.12
SNAPM_BC1_rep	0.05	<0.05	ND	ND	0.03	ND	ND	ND	<0.02	0.12	ND	0.02	ND	0.12
SNAPM_BC2	0.07	<0.05	ND	ND	0.11	ND	ND	ND	<0.02	0.20	ND	0.02	ND	0.20
SNAPM_BC2_rep	<0.04	<0.05	ND	ND	<0.02	ND	ND	ND	<0.02	0.18	ND	0.03	ND	0.18

Table B2 : Analytical PFAS results for samples from Rapid Creek (RC); M – muscle; MUD –Mudcrab; rep – replicate

Composite ID	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFBS	PFHxS	PFOS	PFUnDA	PFDODA	PFDS	PFHxS/PFOS
ng/g - LOR	0.04	0.05	0.03	0.01	0.02	0.02	0.04	0.04	0.02	0.04	0.15	0.08	0.02	
MUDM_RC1	0.14	0.07	0.08	0.03	0.15	0.11	0.10	<0.04	1.10	9.07	0.11	0.32	0.07	10.17
MUDM_RC1_rep	0.12	0.06	0.07	0.03	0.21	0.13	0.12	<0.04	1.36	12.06	0.13	0.53	0.10	13.42
MUDM_RC2	1.10	0.08	0.26	0.08	0.54	0.41	0.20	<0.04	1.52	7.64	0.15	0.59	0.11	9.16
MUDM_RC3	0.15	0.10	0.13	0.04	0.20	0.12	0.09	<0.04	0.42	2.72	0.11	0.47	0.05	3.14

Table B3: Analytical PFAS results for samples from Ludmilla Creek (LD); M – muscle, L – liver, H – hepatopancreas ; MUD –Mudcrab, BARRA – Barramundi, PRAW - Prawn; rep – replicate

Composite ID	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFBS	PFHxS	PFOS	PFUnDA	PFDODA	PFDS	PFHxS/PFOS
ng/g - LOR	0.04	0.05	0.03	0.01	0.02	0.02	0.04	0.04	0.02	0.04	0.15	0.08	0.02	
MUDH_R/LD1	0.47	ND	0.83	0.20	1.69	0.51	0.24	ND	8.78	33.66	ND	**1.07**	ND	42.44
MUDH_R/LD1_rep	0.23	ND	0.67	0.21	1.77	0.51	0.34	ND	8.35	38.62	0.25	1.02	ND	46.97
MUDH_R/LD2	0.80	ND	2.58	1.39	9.23	2.16	**0.83**	ND	40.95	139.16	**0.23**	ND	ND	180.11
MUDH_R/LD2_rep	0.67	ND	2.37	1.20	7.89	2.40	**0.80**	ND	39.00	140.28	ND	ND	ND	179.28
MUDM_LD1	0.14	0.03	0.02	0.01	0.09	0.03	0.04	<0.04	0.26	2.71	0.05	0.28	<0.02	2.97
MUDM_LD2	0.21	0.04	0.00	0.02	0.13	0.04	0.16	<0.04	0.27	0.73	0.13	0.57	<0.02	1.00
BARRAL_LD1	ND	ND	ND	ND	0.31	0.16	0.12	ND	17.20	72.94	ND	ND	ND	90.15
BARRAL_LD1_rep	ND	ND	ND	ND	0.27	0.16	ND	ND	16.87	68.02	ND	ND	ND	84.89
BARRAM_LD2	ND	ND	ND	ND	0.07	ND	ND	ND	1.46	6.30	ND	ND	ND	7.76
BARRAM_LD2_rep	ND	ND	ND	ND	0.04	ND	ND	ND	1.41	6.30	ND	ND	ND	7.71
BARRAL_LD2	ND	ND	ND	0.02	0.59	0.21	**0.21**	ND	26.56	93.74	ND	ND	ND	120.30
BARRAM_LD1	ND	ND	ND	ND	0.04	ND	ND	ND	0.64	2.85	ND	ND	ND	3.49
PRAWM_LD1	ND	ND	ND	<0.01	0.55	0.06	0.11	ND	0.59	40.50	0.12	0.19	ND	41.09
PRAWM_LD2	ND	ND	0.03	<0.01	0.09	0.05	0.10	ND	0.94	40.21	0.17	0.15	ND	41.15
PRAWM_LD2_rep	ND	ND	0.03	<0.01	0.08	0.05	0.10	ND	0.84	37.52	0.13	0.11	ND	38.36
PRAWM_LD3	ND	ND	0.03	<0.01	0.21	0.06	0.12	ND	0.54	48.57	0.12	0.15	ND	49.11
PRAWM_LD3_rep	ND	ND	<0.03	<0.01	0.13	0.05	0.09	ND	0.46	42.98	0.10	0.10	ND	43.44
PRAWM_LD4	ND	ND	0.03	<0.01	0.13	0.05	0.17	ND	0.75	20.88	0.16	0.21	ND	21.63
PRAWM_LD4_rep	ND	ND	0.03	<0.01	0.17	0.04	0.18	ND	0.88	26.21	0.14	0.22	ND	27.09
PRAWM_LD5	ND	ND	<0.03	<0.01	0.19	0.04	0.05	0.08	0.64	6.77	0.08	0.19	ND	7.40
PRAWM_LD5_rep	ND	ND	<0.03	<0.01	0.33	0.04	0.08	0.18	1.01	12.01	0.14	0.34	ND	13.02