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An assessment of per- and poly-fluoroalkyl substances (PFAS) in the surface water and biota of the Swan Canning Estuary and its catchment

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1 Acknowledgments

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2 Summary

Per- and poly-fluoroalkyl substances (PFAS) are an emerging environmental contaminant. The extreme persistence and resistance to biological degradation results in an accumulation of these substances in the environment. In addition, PFAS binds to proteins in the animal tissue, particularly in the blood, liver and gonads, where they are known to bioaccumulate. Animal studies have shown chronic exposure may result in reproductive impairment and liver impacts. In 2016, PFAS were detected in the Swan Canning Estuary and catchment during construction works and separately, a report by the South Australia Environmental Protection Authority detected comparatively high levels in bottlenose dolphins from the Swan Canning Estuary.

Consequently, in December 2016 the Department of Biodiversity, Conservation and Attractions (DBCA) commenced an investigation to ascertain PFAS levels in both surface water and biota within the Swan Canning Estuary and its catchment. Surface water was sampled at 52 estuary and catchment sites, four times over two years (December 2016, 2017, and June 2017, 2018). To explore the potential assimilation into aquatic species, two estuarine resident aquatic species were selected for analysis, black bream and blue swimmer crabs. These two species are regularly targeted by recreational anglers and sufficient samples were selected to calculate robust human consumption guidance.

In the surface water of the Swan Canning Estuary, PFAS were detected at every site above the 99% species protection limit. The major PFAS compounds detected were perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS) and perfluorooctanoic acid (PFOA). The concentrations of PFAS in the estuary were highest in the Middle Swan Estuary and Canning Estuary reflecting the location of significant catchment inputs. In the Swan Canning catchment, the concentrations varied widely from no detections in the Avon River to exceedances of the 90% species protection limit in the Airport North Main Drain.

The sub-catchments with the highest concentrations of PFAS were the Airport North and Airport South Main Drains, Mill Street Main Drain and the Ellen Brook. The 95% species protection limit was exceeded at these sites on at least one occasion. The PFAS compounds detected varied widely within the catchment reflecting the different land uses within each sub-catchment. In particular, the percentage of PFOS and PFHxS to total PFAS in the Perth Airport North and South Main Drains and the Ellen Brook (in June) was indicative of legacy contamination from now disused fluorinated firefighting foams containing long chain PFAS. While PFOS was detected at every site, short chain PFAS including perfluorobutanoic acid (PFBA), perfluorobutane sulfonate (PFBS), perfluoropentanoic acid (PFPA) and 6:2 fluorotelomer sulfonate (6:2 FTS) were also commonly detected.

Concentrations of PFOS+PFHxS (PFOS and PFHxS combined) in the surface water of the estuary did not exceed the recreational guideline related to recreational activities in the estuary. The PFOS+PFHxS concentrations in the Airport North drain, however, exceeded the recreational guideline however as the drain in this region is

listed as a *Contaminated site-Restricted Use,* the drain is not the focus of recreational activity and so the potential for exposure is minimal.

In the biota, PFAS were detected in all tissue types in both the black bream (muscle, liver, gonads and carcass) and blue swimmer crab (muscle and hepatopancreas). The concentration of PFAS were the lowest in the muscle and highest in the liver and hepatopancreas in the bream and crabs respectively. A comparison with data from estuaries around Australia suggested that the concentrations detected in this study were not unusual in an urban estuary with a history of PFAS exposure. The consumption guidance calculated for bream fillets and crab muscle determined that a restriction of black bream or blue swimmer crab consumption due to PFAS contamination is not required. This assessment is supported by the Human Health Risk Assessment completed by the Department of Health (DOH) and available on the DBCA and DOH websites.

3 Introduction

3.1 Background

The presence of per- and poly-fluoroalkyl substances (PFAS) in the environment is a significant emerging issue nationally. Since the 1970's, PFAS have been used in an extensive range of industrial and consumer products including carpets, leather treatments, kitchenware coatings, outdoor clothing, mist suppressants used in metal plating and firefighting foams (Kotthoff et al. 2015). The widespread use of these compounds is due to a range of useful properties, including water repellence when woven as fabric or as fabric treatment, non-stick surfaces through water and lipid repellence, and stain, heat and chemical resistance. The potential environmental impact from PFAS contamination is due to their stability and resistance to biological and chemical degradation, combined with its solubility in water. Furthermore, long chain PFAS compounds comprising greater than six carbon atoms are known to bioaccumulate in biota and include the previously widely used compounds perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorooctanoic acid (PFOA) and. In contrast to a range of other organic contaminants, such as organochlorine pesticides, PFOS, PFHxS and PFOA preferentially bind to proteins rather than lipids, in particular blood albumin (Jones et al. 2003). Consequently, the highest concentrations are generally found in animal blood and liver (Martin et al. 2003). A range of observed chronic health effects have been identified in animal test subjects and generally include liver and reproductive health impacts (Kyunghee et al. 2008, Nordén et al. 2016, Lee et al. 2017, Chen et al. 2018).

In recognition of these environmentally persistent and bioaccumulative properties, global action on the control of these compounds is occurring. In particular, PFOS has been recognised as a persistent organic pollutant and in 2010 was listed under Annex B (restriction of use) of the Stockholm Convention. In addition, PFOA has been reviewed and in May 2019 it was added to Annex A (elimination of production and use) of the Convention, and the review process has commenced for PFHxS (United Nations Environment Program 2019). Globally, more than 151 counties have ratified the amendment to Annex B of the Stockholm Convention. Of importance, China, the world's largest producer of PFAS, ratified the amendment in 2014 and while the country is still producing PFOS, it is working to reduce the use and production of PFOS and the eventual phase out of this compound in priority sectors (Tian 2016). The United States of America, previously one of the world largest manufactures of PFOS and PFOA, has taken steps to control and reduce the manufacture and use of PFOS and PFOA (United States Environmental Protection Agency 2019).

Despite its position as a signatory to the Stockholm Convention, Australia is yet to ratify the amendment adding PFOS to Annex B. However, the process for the formal ratification of the amendment has commenced with the Australian Government having released a Regulation Impact Statement for public consultation, which concluded in February 2018 (Department of Agriculture Water and the Environment

2020). In recognising the need for a coherent and consistent national PFAS management strategy, all Australian States and Territory Governments have developed the National Environmental Management Plan (HEPA 2018). Additionally, there is the ongoing development of the National Standard for Environmental Risk Management of Industrial Chemicals which when in operation in 2022 is expected to provide controls on the use of harmful PFAS. However, as yet, no comprehensive nationwide regulation exists to control the use of harmful PFAS. There has however, been a gradual reduction and phase out of harmful PFAS in some key industries, primarily its use in firefighting foams (termed aqueous film foaming foams – AFFF). The Australian Department of Defence phased out the use of PFOS in its AFFF by 2007 (Department of Defence 2007), and Air Services Australia, who manage most Australian civil airports, stopped using PFOS or PFOA based AFFF in 2003 and phased out all PFAS containing foams by 2010. However, Department of Defence and Air Services Australia have identified that legacy PFAS contamination may pose an ongoing environmental and human health risk at many of their airbases and airports. Consequently these organisations are currently conducting detailed site investigations to determine the extent of potential contamination (Airservices Australia 2019, Department of Defence 2019).

In Western Australia, the Department of Biodiversity, Conservation and Attractions (DBCA) was made aware of potential sources of PFAS to the Swan Canning Estuary in 2016. Initial detections arose from site investigations associated with the Forrestfield-Airport Link project. Surface water and ground water samples taken in the vicinity of the project site, west of the airport and in close proximity to the estuary displayed elevated concentrations of PFAS, particularly PFOS. The recent site investigations of the Perth Airport lease by Perth Airport Pty Ltd and Air Services Australia also identified PFAS contamination (Ascot 2018, Senversa 2019). In addition, the Australian Department of Defence site, Pearce Airbase, is likely to be a significant source of PFAS to the estuary after a detailed site investigation revealed significant PFAS contamination (GHD 2018). Furthermore, a recent report by the South Australian EPA (Gaylard 2017) compared the PFAS concentrations in liver samples of dolphins from jurisdictions around Australia, including the Swan Canning Estuary. Samples from Swan Canning Estuary dolphins displayed the highest concentrations of PFAS, in particular PFOS, of all samples analysed in the study (Gaylard 2017).

Given the number of potential sources of PFAS contamination within the Swan Canning Estuary catchment and the current evidence indicating elevated concentrations of PFAS in dolphins, an investigation into the current extent and distribution of PFAS contamination in the Swan Canning Estuary and catchment was required.

3.2 Scope

To understand the potential risk of PFAS contamination to the ecological health of the Swan Canning Estuary and its catchment this project investigated the extent and distribution of PFAS in surface water in the estuary and its catchment. Given the importance of recreational fishing to the local population, human consumption

guidance was also determined for two key recreational species, black bream (*Acanthopagrus butcheri*) and blue swimmer crabs (*Portunas armatus*), following the method outlined in Hoeksema (2015). Black bream and blue swimmer crabs occupy different ecological niches within the estuary, are estuarine residents and recent studies have found that the exposure and accumulation of PFAS in crabs and fish can differ widely (e.g. Vijayasarathy et al. 2017, Taylor et al. 2018). On this basis, these two species were selected for analysis in this study. In addition, PFAS has been shown to bind preferentially to proteins, particularly blood albumin (e.g. Martin et al. 2003) and to gonad tissue during maturation (e.g. Sharpe et al. 2010), and thus it was expected that PFAS may be differentially partitioned in tissue types of black bream and blue swimmer crabs.

The major aims of this study were to:

- Determine the extent and distribution of PFAS in surface water of the Swan Canning Estuary and its catchment.
- Investigate the potential environmental impact of PFAS contamination in the Swan Canning Estuary and its catchment, by;
    - Comparing surface water concentrations against the draft Australian Water Quality Guidelines (Heads of EPA's Australia and New Zealand - HEPA 2018), and
    - Determining the concentrations of PFAS in different tissue types (muscle, liver, gonads and/or carcass) in black bream and blue swimmer crabs; and
- Determine consumption guidance for recreational fishers for black bream and blue swimmer crabs.

Further investigation of PFAS contaminants in dolphins was not part of this investigation. The DBCA is currently working collaboratively with Murdoch University to understand dolphin health and the findings of that work are anticipated for release in 2020.

4 Materials and Methods

4.1 Site description

The Swan Canning Estuary is a large microtidal estuary situated wholly in the Perth Metropolitan Region. Major freshwater inputs into the system are primarily from the local catchment. The majority of large tributaries draining to the estuary, excluding the Avon River and Ellen Brook, have been dammed for the provision of municipal water supply. The Avon River and Ellen Brook are the major unregulated tributaries and can provide significant flow to the system. During the late spring, summer and early autumn, the estuary is tidally dominated, and the salt wedge slowly migrates upstream causing significant stratification and often anoxia in the depths (Huang et al. 2018). During the winter period freshwater flows push the salt wedge downstream into the lower estuary and freshwater/brackish conditions become prevalent in the mid to upper reaches. The lower estuary remains marine to brackish throughout the year.

As a result of the differing environmental conditions throughout estuary system, the DBCA recognises five distinct ecological management zones (EMZ) in the Swan Canning Estuary (Swan River Trust 2009). These regions are the Lower Swan Canning Estuary (LSCE), the Middle Swan Estuary (MSE), the Upper Swan Estuary (USE), the Canning Estuary (CE), and the Lower Canning River (LCR) (Figure 1). Site names, site codes and respective EMZ have been provided in Table 1.

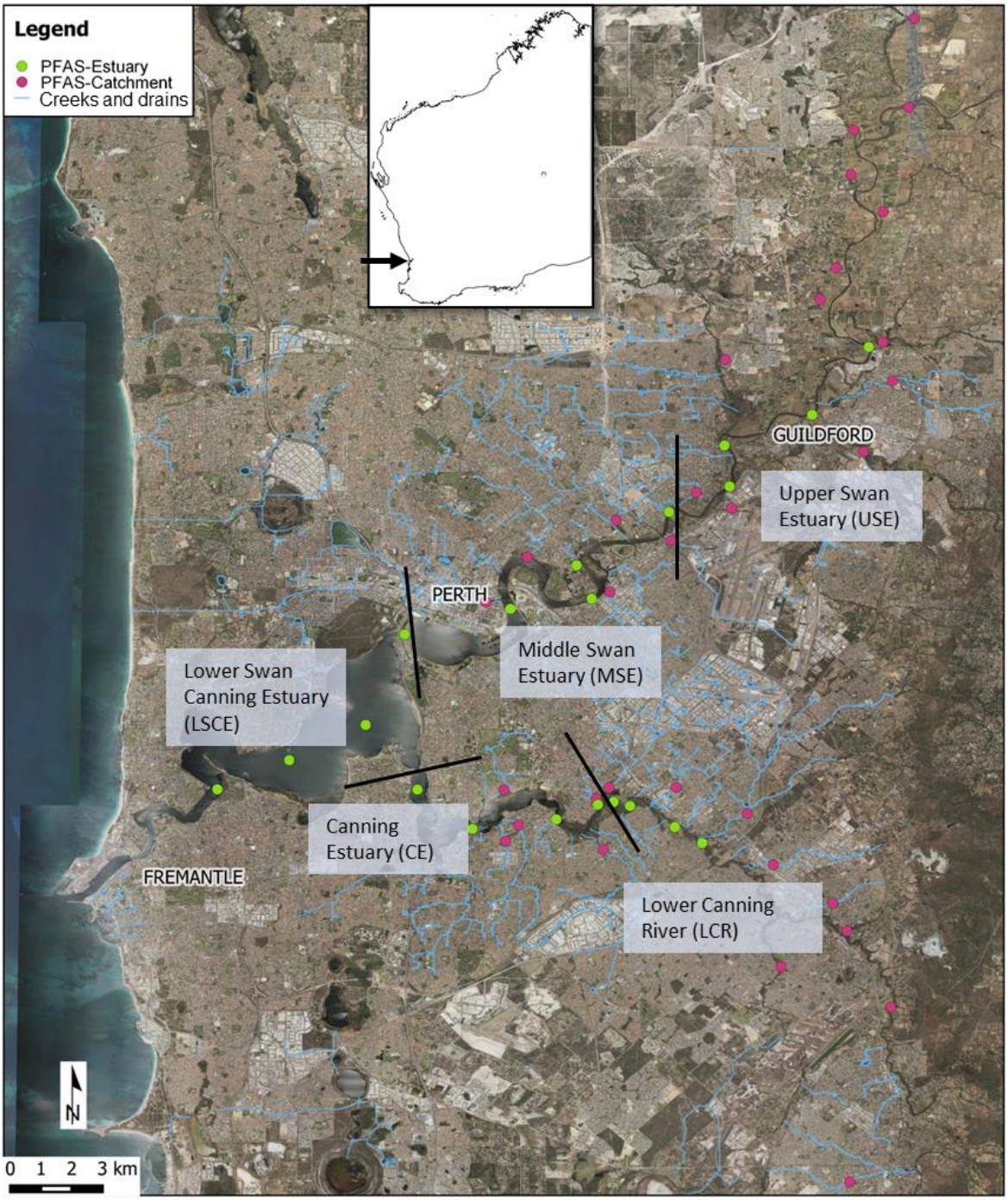

Figure 1. Ecological management zones in the Swan Canning Estuary (SRT, 2009). Surface water sampling sites and creek and drainage lines are included on the map for reference. Arrow in insert map shows the location of the Swan Canning Estuary in Western Australia

Table 1. Surface water sampling sites (codes, site names) within and draining into each estuary Ecological Management Zone (EMZ).

Within the Swan Canning Estuary			Catchment sites draining to the Swan Estuary			Catchment sites draining to the Canning Estuary		
EMZ	Site code	Site name	EMZ	Site code	Site name	EMZ	Site code	Site name
Lower Swan Canning Estuary	BLA	Blackwall Reach	Middle Swan Estuary	CB13	Claise Bk Main Drain	Canning Estuary	SCCIS1	Galway Road
	ARM	Armstrong Spit		MIMDOUT	Maylands Main Drain		SCCIS2	Holmes Street
	HEA	Heathcote		SWS13	Sth Belmont Main Drain		BAMDKD	Beatrice Ave Main Drain
	NAR	Narrows Bridge		SWS10	Bayswater Main Drain		WIFRD	Wilson Main Drain
Middle Swan Estuary	NIL	Nile Street		KANAV	Airport Sth Main Drain		SWS1	Mill St Main Drain
	STJ	St John's Hospital		CSMDREID	Chapman st Main Drain	Lower Canning River	SWS2	Bannister Creek
	MAY	Maylands Pool		SCCIS12	Airport Nth Main Drain		SCCIS3	Lower Canning
	RON	Ron Courtney Island		SWN10	Helena River		SWS3	Yule Brook
Upper Swan Estuary	KIN	Kingsley Drive		SWN12	Bennet Brook		SWS4	Bickely Brook
	SUC	Success Hill		SWN8	Blackadder Creek		SWS7	Southern River
	WMP	West Midland Pool	Upper Swan Estuary	SWN7	Jane Brook		SCCIS4	Helm St Main Drain
	MSB	Middle Swan Bridge		WNDCK	Wandoo Creek		EBGS01	Ellis Brook
Canning Estuary	SCB2	South Canning Bridge		SCCIN3	St Leonards Creek		SWS12	Upper Canning River
	SAL	Salter Point		SWN11	Susannah Brook		AW05	Southern River
	RIV	Riverton		HBBROCK	Henley Brook			
	CASMID	Castledare		SWN9	Ellen Brook Downstream			
Lower Canning River	KEN	Kent St Weir		SWN3	Ellen Brook Upstream			
	BAC	Bacon Street		SWN5	Avon River			
	NIC	Nicholson Rd Bridge						
	ELL	Ellison Parade						

4.2 Surface Water

To assess the extent and distribution of PFAS within the Swan Canning Estuary, surface water samples were collected at 20 sites throughout the estuary (Table 1 & Figure 2) every six months over a two-year period, specifically December 2016, June 2017, December 2017 and June 2018. The sampled sites provided thorough spatial coverage of the entire estuary and are regularly sampled as part of the routine DBCA water quality monitoring program.

Sampling within the catchment of the Swan Canning Estuary targeted 32 sites on the same time period as the estuary sampling (Table 1 & Figure 2). The catchment sites represented the major surface water inflows into the estuary and are also sampled by DBCA as part of the routine catchment monitoring program. Three sites regularly sampled as part of the DBCA routine catchment monitoring program (Susannah Brook, Wandoo Creek, and Ellis Brook) were not flowing during any of the four sampling periods and consequently were not included in this study. The Ellen Brook was sampled at two locations. Ellen Brook Downstream (DS) was located near the confluence with the USE and flows perennially. Ellen Brook Upstream (US) was located approximately 7 km further upstream at the Department of Water and Environmental Regulation hydrometric gauging station. This section of the river flows seasonally through winter and spring. In the drier months, when Ellen Brook US was not flowing, the results obtained from Ellen Brook DS likely represent flow from the lower section of the catchment (i.e. below Ellen Brook US). However, when Ellen Brook US was flowing, data collected at both sites likely represented flows from the wider catchment, including the Pearce Airbase.

Cross contamination was a significant risk in the sampling for PFAS in surface waters. To avoid cross contamination the guidance developed by the Western

Australian Department of Environment Regulation (Department of Environment Regulation 2016) (now Department of Water and Environmental Regulation (DWER)) were strictly adhered to. Details of the sampling procedure are provided in Appendix A.

At each site, after the water sample was collected, physiochemical variables were measured within 20 cm of the surface using a YSI ProDSS handheld water quality multiprobe in the catchment and in the estuary a YSI EXO2 multiprobe was used, both calibrate prior to and post use.

Water samples were analysed for the DWER recommended suite of 10 compounds (Department of Environment Regulation 2016) at the lowest available limits of reporting (Table 2). Biota samples were analysed for an expanded suite of 14 analytes inclusive of the DWER recommend suite. Samples were analysed by a NATA accredited laboratory at the lowest limit of reporting available (Table 2).

Table 2. List of targeted PFAS compounds and the laboratory provided limit of reporting.

Compound	**Abbreviation**	**Water**	**LOR (µg/L)**	**Biota**	**LOR (mg/kg)***
Perfluorobutanoic acid	PFBuA	Y	0.002	Y	0.001
Perfluoropentanoic acid	PFPeA	Y	0.0005	Y	0.0005
Perfluorohexanoic acid	PFHxA	Y	0.0005	Y	0.0005
Perfluoroheptanoic acid	PFHpA	Y	0.0005	Y	0.0005
Perfluorooctanoic acid	PFOA	Y	0.0003	Y	0.0003
Perfluorononanoic acid	PFNA	N	N/A	Y	0.0005
Perfluorodecanoic acid	PFDA	N	N/A	Y	0.0005
Perfluoroundecanoic acid	PFUdA	N	N/A	Y	0.0005
Perfluorododecanoic acid	PFDoA	N	N/A	Y	0.0005
Perfluorobutane sulfonic acid	PFBS	Y	0.0005	Y	0.0005
Perfluorohexane sulfonic acid	PFHxS	Y	0.0005	Y	0.0005
Perfluorooctane sulfonic acid	PFOS	Y	0.0003	Y	0.0003
6:2 Fluorotelomer sulfonic acid	6:2 FTS	Y	0.001	Y	0.0005
8:2 Fluorotelomer sulfonic acid	8:2 FTS	Y	0.001	Y	0.0005

* Due to the complexity of the biota sample matrices, particularly the crab hepatopancreas, some detection limits varied from those specified below Table 1. This variation has been noted in the relevant section.

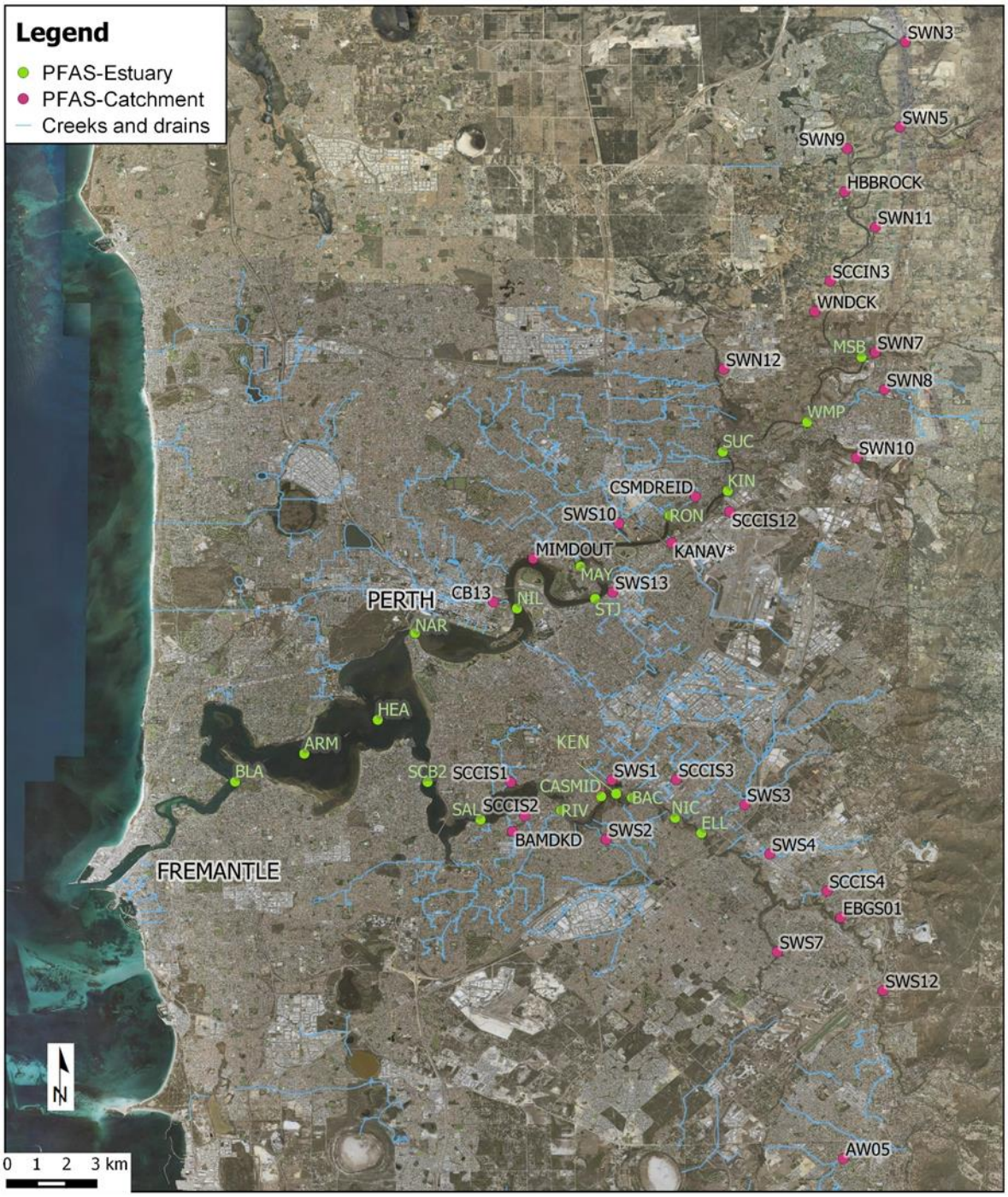

Figure 2. Surface water sampling sites within the Swan Canning Estuary and catchment. Major drainage lines are shown on the map

4.3 Biota

For the development of robust consumption guidance 30 black bream (*Acanthropagrus butcheri*) and 30 blue swimmer crabs (*Portunas armatus*) were collected and retained for analysis. To ensure the results reflected likely exposure to PFAS from consumption of fish or crabs by recreational fishers, all specimens retained were above the respective minimum legal limit for each species. Both bream and crab samples were analysed by a NATA accredited laboratory.

4.3.1 Black bream

Black bream (*Acanthopagrus butcheri*) were collected from the Middle Swan Estuary on 27 November 2017 and the Canning Estuary on 30 November 2017 using a 41.5 m seine net with 25 mm mesh size and 2 m vertical drop (Figure 3). The net swept an area of 274 m^2 and was laid in a semi-circle from the bank by boat and then hauled onto the beach. The larger *A. butcheri* captured were placed in a holding tank (pre-cleaned with ethanol, rinsed with deionised water and filled with site water), while bycatch and juvenile *A. butcheri* were immediately returned to the estuary. Fish were then measured and those greater than the minimum legal length (250 mm total length) were placed into an ice bath.

4.3.2 Blue swimmer crabs

Blue swimmer crabs (*Portunas armatus*) were sampled in the CE, MSE and the LSCE (Figure 3). Traps were set on 11 December 2017 and retrieved the follow day, allowing for a minimum of a 24-hour deployment time. Trapping effort consisted of three traps at each of four sites within the MSE and the CE, and at five sites within the LSCE (Figure 1). A total of 39 traps were set throughout the estuary. Traps were an hourglass configuration with a diameter of 1,150 mm and a standing height when set of 550 mm (Harris et al 2016).

Traps were baited with yellow eyed mullet (*Aldrichetta forsteri*) sourced from the Peel Harvey Estuary. Baits were inserted into a perforated PVC tube to allow the scent of the bait to disperse throughout the water and attract crabs, while ensuring the bait could not be consumed. All handling of the baits and bait tubes were done with clean nitrile gloves. The risk of PFAS contamination from the bait and traps was considered low due to the long deployment time in the water during sampling and the general cleanliness of the trap.

The traps were hauled by hand onto the boat. Once the trap was at the water surface it was gently shaken in the water to remove any debris and emptied directly into an ice bath. Once the crabs were anaesthetised, they were measured to ensure they were above the minimum legal limit (127 mm CW), sexed, double bagged (in food grade HDPE snaplock bags) and euthanised by placing on ice (double bagged) in a clean storage esky (washed with ethanol and rinsed with deionised water) and transported to the laboratory for processing. Fish, juvenile crabs and other bycatch were returned live to the estuary.

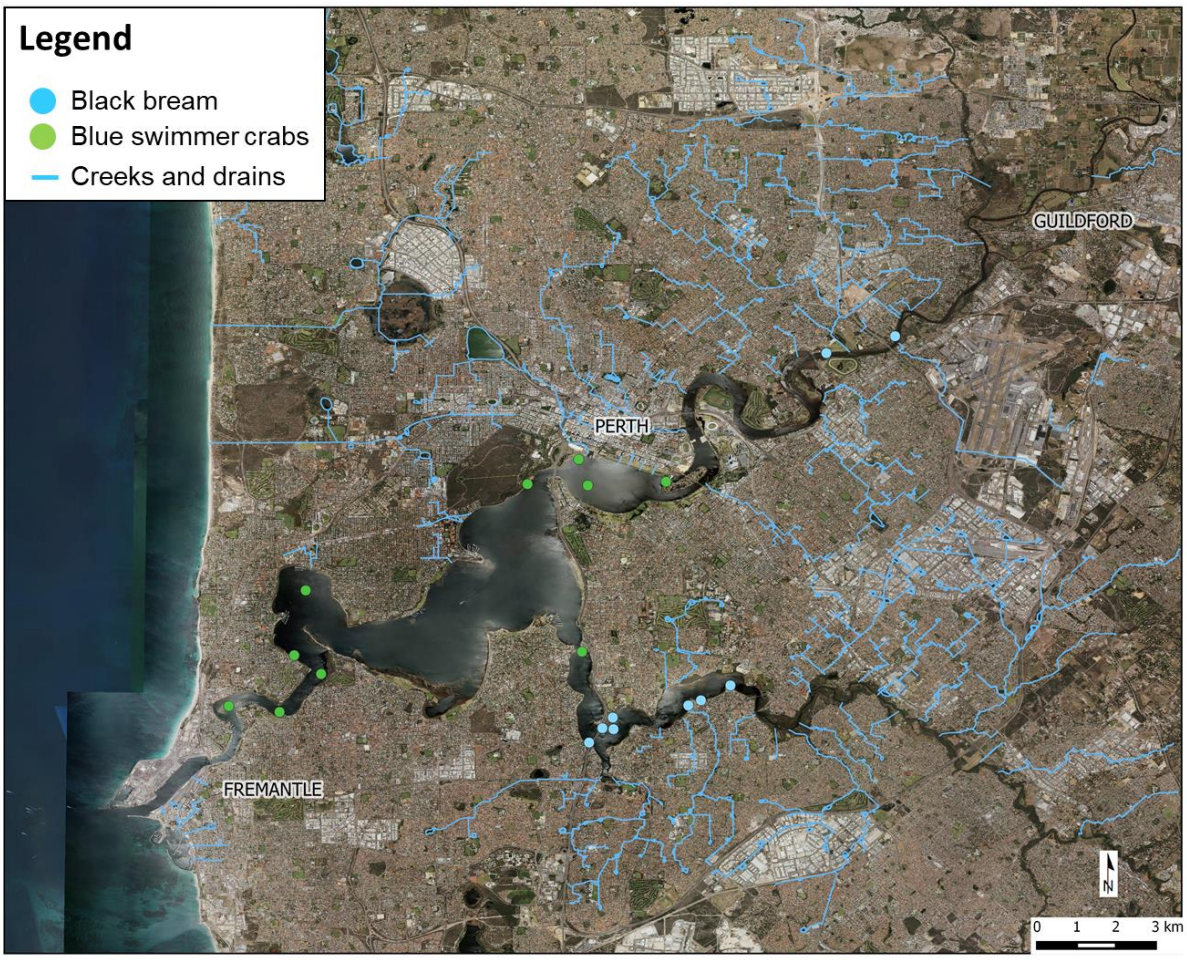

Figure 3. Sites where black bream and blue swimmer crabs were captured in the Swan Canning Estuary. Sites were sampling was attempted but not successful are not shown.

4.3.3 Biota processing

Black bream

Each of the retained fish was weighed to the nearest 0.01 g, the total length measured to the nearest millimetre and were sexed. Fish were then scaled using a cleaned stainless-steel knife and both fillets removed, rinsed with deionised water (to remove scales and other debris), dried with a clean paper towel, weighed and frozen. One fillet was submitted to the laboratory for analysis while the other was retained for potential future analysis. The gonads and liver were then removed, rinsed with deionised water, dabbed dry with a clean paper towel and weighed to 0.01 g. Finally, the carcass, including the stomach and gonads, was weighed and frozen. Thirty frozen samples of each tissue type were delivered to the laboratory for analysis. In addition to the fillet, liver and carcass samples, six gonad samples, three male and three females, were retained separately from six specimens from the Middle Swan Estuary to examine potential PFAS partitioning within these organs.

Blue swimmer crabs

Crabs were sexed according to the appearance of the abdominal flap (Potter and de Lestang 2000). Carapace width (distance between the lateral spines of the carapace) was measured to the nearest millimetre and total weight was measured to the nearest 0.01 g. Muscle tissue from the chelipeds and pereopods (walking and swimming legs) was extracted and frozen for analysis. The viscera, including the gills, hepatopancreas and any remaining internal organs, was carefully removed from the carapace. Care was taken to ensure organs were intact and the inclusion of muscle tissue from within the cephalothorax was minimised. Thirty muscle and thirty viscera samples were then frozen before delivery to the laboratory for analysis

4.4 Data analysis

4.4.1 Surface water

Surface water data from each site and sampling occasions were initially compared to the relevant draft Australian Water Quality Guidelines (AWQG) guidelines for species protection (Table 3). It must be noted that the lowest limits of reporting available from the laboratory for PFOS was higher than the AQWG for 99% species protection, thus any detect of PFOS is considered and exceedance of this guideline. The 99% species protection is usually reserved for high conservation value systems, with the 95% species protection more generally applied to slightly to moderately disturbed systems such as the Swan Canning Estuary. However, for bioaccumulative toxicants such as PFOS the application of the 99% protection level is recommended in order to account for secondary effects (Dept of Environment and Energy, 2016). Additionally, the potential land uses or activities that resulted in PFAS contamination may be indicated by the composition of total PFAS (summed) in the contaminated media (soil or water). In the absence of major PFAS manufacturing centres, if the PFAS composition is dominated by PFOS and PFHxS, then historical use of aqueous film forming foam for firefighting may be suspected (e.g. Ahrens et al. 2015). For this reason the percent contribution of PFOS+PFHxS to total PFAS has been calculated.

To examine spatial and temporal trends in the concentration of PFAS compounds in surface waters in the Swan Canning Estuary, data were analysed using PERMANOVA and nonmetric multidimensional scaling (nMDS) in the statistics package PRIMER (Version 7, PRIMER-E, Plymouth). The EMZs were utilised in the analysis of both the estuary and catchment data. The estuary sampling consisted of the collection of four samples (sites) in each of the regions on each sampling period to facilitate the calculation of a regional mean concentration (Figure 2). The catchment sites have been grouped according to the estuary region in which the drain or stream confluence is situated. All the PFAS compounds detected were used in the analysis (the compound 8:2 FTS was not detected at any site and thus not used in the analysis). The data were firstly square root transformed to reduce the

impact of very high values on the analysis, before a Euclidean Distance resemblance matrix was constructed. A two-way crossed PERMANOVA was used to determine the effect of EMZ (five levels) and sampling periods (four levels) on PFAS concentrations and any interaction. Where a significant effect was revealed, the estimated components of variation were used to assess the factor (or combination) responsible for most variation in the analysis. The data were further explored using nMDS. The analysis of catchment data was conducted in the same manner except the data were fourth root transformed due to the very high concentrations at two catchment sites, and the number of regions was reduced to four as none of the sampled catchments drained directly into the Lower Swan Canning Estuary.

Of the 29 sites (out of 32 visited sites) where a sample was collected, 13 had a currently or previously operating stream gauge (operated by DWER or the Water Corporation). In the context of this study, load was calculated as the quantity of PFAS per hour (mg(PFAS)/hr) and was only relevant to the period of sampling. This calculation of PFAS load provides additional information on the degree to which particular sub-catchments act as a potential PFAS source to the estuary. Where gauging data was available for the sampling period, the load (mg(PFAS)/hr) was calculated using the mean hourly discharge recorded (m^3/s) at the time of sampling. Where a stream gauge was no longer operating, historical data was used to estimate the loads. On these occasions the hourly discharge was calculated by firstly determining the median daily discharge from the previous two years of data from the corresponding month. This value was divided by 24 to give an estimate of the hourly discharge and then used to calculate load (mg(PFAS)/hr). Where there was no stream gauging information for a site, PFAS load could not be determined for the catchment. In June 2018 the Yule Brook gauging station was not operable, thus load was calculated for this time period using the approach described above.

Table 3. Relevant guidelines used in this report. Guideline values are taken from the PFAS National Environmental Management Plan (HEPA 2018) and are based on the Draft Water Quality Guidelines and health based guidelines were sourced from the Department of Health (2019).

Draft Guideline	PFOS	PFOA	PFOS+PFHxS
99% species protection (µg/L)	0.00023	19	
95% species protection (µg/L)	0.13	220	
90% species protection (µg/L)	2.0	632	
80% species protection (µg/L)	31	1 824	
Recreational water quality guideline (µg/L)		10.0	2.0
Dietary consumption guidelines – tolerable daily intake (µg/kg-body weight/day		0.16	0.02

4.4.2 Biota

The statistical analysis of PFAS in biota tissues focused on the concentration of PFOS+PFHxS. This approach was taken because these two compounds were the dominant compounds detected in biota in this study, the human health and ecological effects of these two compounds are thought to be similar (enHealth 2019) and it is an approach used by others (Taylor et al. 2018).

A three-way analysis of variance (ANOVA) was used to examine the difference in PFOS+PFHxS concentrations across sex (male and female), region (Middle Swan Estuary and Canning Estuary) and tissue type (muscle, liver, carcass) in *A. butcheri*. The data were log transformed to meet the assumptions of ANOVA.

To further explore the tissue partitioning of PFAS in *A. butcheri* during reproductive development, six gonad samples were obtained from three male and three female fish from the Middle Swan Estuary. A two-way ANOVA was undertaken to determine if tissue PFOS+PFHxS concentrations varied between tissue types (gonads, fillet, liver, carcass) and sex. Data were log transformed prior to the analysis.

Due to a lack of sufficient regional samples for *P. armatus*, a two-way ANOVA was used to examine the difference in concentrations between sex (male and female) and tissue type (muscle and viscera). The data were log transformed prior to ANOVA.

Regression analysis was used to explore the relationship between fish length and PFOS+PFHxS concentration and body burden. Firstly, total fish PFAS body burden was calculated using the following equation:

$$\mathrm{WFl} = (\mathrm{cM} \times \mathrm{wM}) + (\mathrm{cL} \times \mathrm{wL}) + (\mathrm{cC} \times \mathrm{wC})$$

Whole fish PFOS+PFHxS concentration was calculated as:

$$\mathrm{WFc} = \mathrm{WFl} / [\mathrm{wM} + \mathrm{wL} + \mathrm{wC}]$$

With WFl = whole fish PFOS+PFHxS load, WFc = whole fish PFOS+PFHxS concentration, c = concentration of PFOS+PFHxS (µg/g), w= weight of tissue (g), M = muscle, L = liver and C = carcass. Both concentration and body burden were log transformed to improve the agreement with the assumptions of linear regression.

To further investigate the accumulation of PFAS in body tissues and potential impacts on fish health the relationship between PFOS+PFHxS and gonadosomatic index (GSI) and hepatosomatic index (HSI) were tested. The GSI and HSI were calculated using the follow equations:

GSI = (GW/TW) x 100.

HSI = (LW/TW) x 100.

With GW = gonad weight (g), TW = total weight (g) and LW = liver weight. Regression analysis was used to examine the relationship between carapace width and tissue concentration and body burden in *P. armatus*. To achieve the best fit of the data with the assumptions of linear regression the concentration and load data were log transformed. In each case, the carapace data were not transformed.

4.4.3 Consumption guidance

Consumption guidance was calculated separately for each species and was based on the mean concentration (C_m) of each contaminant in the muscle tissue of each species. The mean concentration was calculated from all 30 samples and for the purposes of this report, was considered representative of *A. butcheri* and *P. armatus* in the entire Swan Canning Estuary rather than of a specific site or region. For those analytes where non detects were recorded for some samples, the mean concentration was calculated by treating non-detects as half of the limit of reporting for that analyte (United States Environmental Protection Agency 2000 (USEPA)). The same data were also employed for the calculation of the standard error of the mean (C_{SE}).

The Australian Government Department of Health have provided tolerable daily intake limits (TDI) for PFOS+PFHxS and PFOA (Table 3) (Department of Health 2019). These TDIs have been used in the calculation of consumption guidance based on the mean concentration of PFOS+PFHxS and PFOA in the muscle tissue of *A. butcheri* and *P. armatus* in the Swan Canning Estuary. The TDI values were employed to calculate the acceptable number of meals that can be consumed monthly over an entire lifetime by an average person without an appreciable risk to human health. This approach is widely accepted for use with wild-caught food commodities, such as recreational fishing species, and has been adopted by numerous authorities worldwide. Following the methods of the USEPA (2000), a daily consumption limit (CR_{lim}) was first calculated using the equation:

CR_{lim} (kg d^{-1}) = TDI x BW / C_m (USEPA, 2000)

where TDI = the tolerable daily intake (µg/kg-bw – see Table 2), BW = a standardised body weight (kg) of the consumer (males and females combined) and C_m = the mean concentration of the contaminant (µg kg^{-1}). For the current consumption guidance, BW was considered to be 78.5 kg and was based on an average weight of an Australian male (18 years and over) of 85.9 kg and of a female of 71.1 kg (Australian Bureau of Statistics 2012).

The daily consumption limit was then employed to produce an acceptable number of meals of muscle that can be consumed monthly over an entire lifetime without an appreciable risk to human health from non-carcinogenic effects using the formula:

CR_{mm} (meals/month) = CR_{lim} x T_{ap} / MS (USEPA, 2000)

where CR_{mm} = meal consumption limit, T_{ap} = the number of days in the selected period, *i.e.* 30.44 days for a month, and MS = a standardised meal size of 0.150 kg for fish and 0.075 kg for crustaceans (standardised meal sizes from (Food Standards Australia New Zealand 2007).

The USEPA (2000) considers consumption of a food commodity, such as wild-caught fish, to be unrestricted when CR_{mm} > 16 meals/month. The current consumption guidance has also adopted this benchmark.

5 Results

5.1 Surface water – Estuary

Per- and polyfluoroalkyl substances were detected at all routine monitoring sites throughout the Swan Canning Estuary. Of the ten compounds tested in this study, only PFOS and PFHxS were detected at all sites and on all sampling occasions (Figure 4). The concentrations of PFOS ranged from 0.0041 µg/L in June 2017 at BLA (Blackwall Reach) in the Lower Swan Canning Estuary (LSCE) to 0.120 µg/L at CASMID (Castledare) in the Canning Estuary (CE). The concentration of PFHxS ranged from 0.0022 µg/L in June 2017 at BLA (Blackwall Reach) in the LSCE to 0.0510 µg/L in December 2017 at RON (Ron Courtney Island) in the Middle Swan Estuary (MSE). The detection of PFOS at every estuary site (LOR 0.0003 µg/L PFOS) resulted in the exceedance of the draft Australia Water Quality Guidelines (AWQG) for 99% species protection (0.00023 µg/L PFOS) throughout the estuary. The 95% species protection guideline (0.13 µg/L PFOS) was not exceeded at any estuary site, nor was the human recreational water quality guideline (2.0 µg/L PFOS+PFHxS) (Figure 4). The draft AWQG 99% species protection guideline (19 µg/L) for PFOA was not exceeded at any site.

The concentration and composition of PFAS in the estuary showed a significant interaction effect between EMZ and sampling period (2-way interaction: $F_{12, 60} = 1.556$, $p = 0.046$). Clear differences between the EMZs were present (Figure 4). In both the Canning and Swan Estuary the concentrations were low at the most upstream sites (USE and LCR), before increasing along a downstream gradient through the middle reaches of the respective estuaries (MSE and CE), before once again declining in the LSCE (Figure 4, Table 4). Additionally, the contribution of PFOS and PFHxS to total PFAS (summed) differed between the EMZs. The greatest mean PFOS+PFHxS contribution to total PFAS (summed) was observed in the MSE where it consistently contributed between 65-76% of the total. The upstream and downstream EMZ's (USE and LSCE respectively) were similarly dominated by PFOS+PFHxS, where the contribution ranged from 47% in the USE in December 2017 to 78.7% in the USE, in December 2016. PFOS+PFHxS in the Canning River and Estuary (LCR and CE) was much less dominant, ranging from 31.6% in the LCR in December 2017 to a maximum of 67.1% in the CE in December 2016. The short chain compounds including PFPA, 6:2 FTS, PFBS where much more prevalent in the Canning River and Estuary sites (Figure 4).

The variation (range) in concentration of PFAS was typically greatest in December while in June the concentrations were typically lower and more consistent throughout the system (Figure 4, Table 4). The patterns of temporal variation within each EMZ was primarily driven by two major occurrences. Firstly, a clear separation of December 2017 from the remaining sampling periods in each EMZ (Figure 5), caused primarily by the ubiquitous detection of PFBA throughout the estuary (Figure 4 and 5). The much greater variation in the December 2017 data was clearly visible in the width of the horizontal spread (along the x-axis) of data points, which was greater than any other sampling period. The variability along the x axis was driven by

PFOS, PFHxS, 6:2 FTS and PFBS (Figure 5). In June 2018, there was much lower variation in the PFAS composition and concentration across all EMZs in the Swan Canning Estuary. Lower concentrations of key compounds in the MSE, USE, LCR and CE and higher concentrations in the LSCE were evident on that sampling occasion (Figure 4, Table 4).

Estuary water quality data clearly reflects the influence of the tidal marine penetration into the estuary. The LSCE had a high salinity and pH, reflective of the region's proximity to the ocean and the influence of tidal forcing pushing saline water into the estuary (Table 5). The CE and MSE show very similar salinities reflecting their similar upstream position within the estuary. The LCR was dominated by freshwater conditions due to riverine inputs and the tidal barrier of the Kent Street Weir that separate the LCR from the estuarine CE. A higher pH in the LCR may be reflective of algal growth within the LCR.

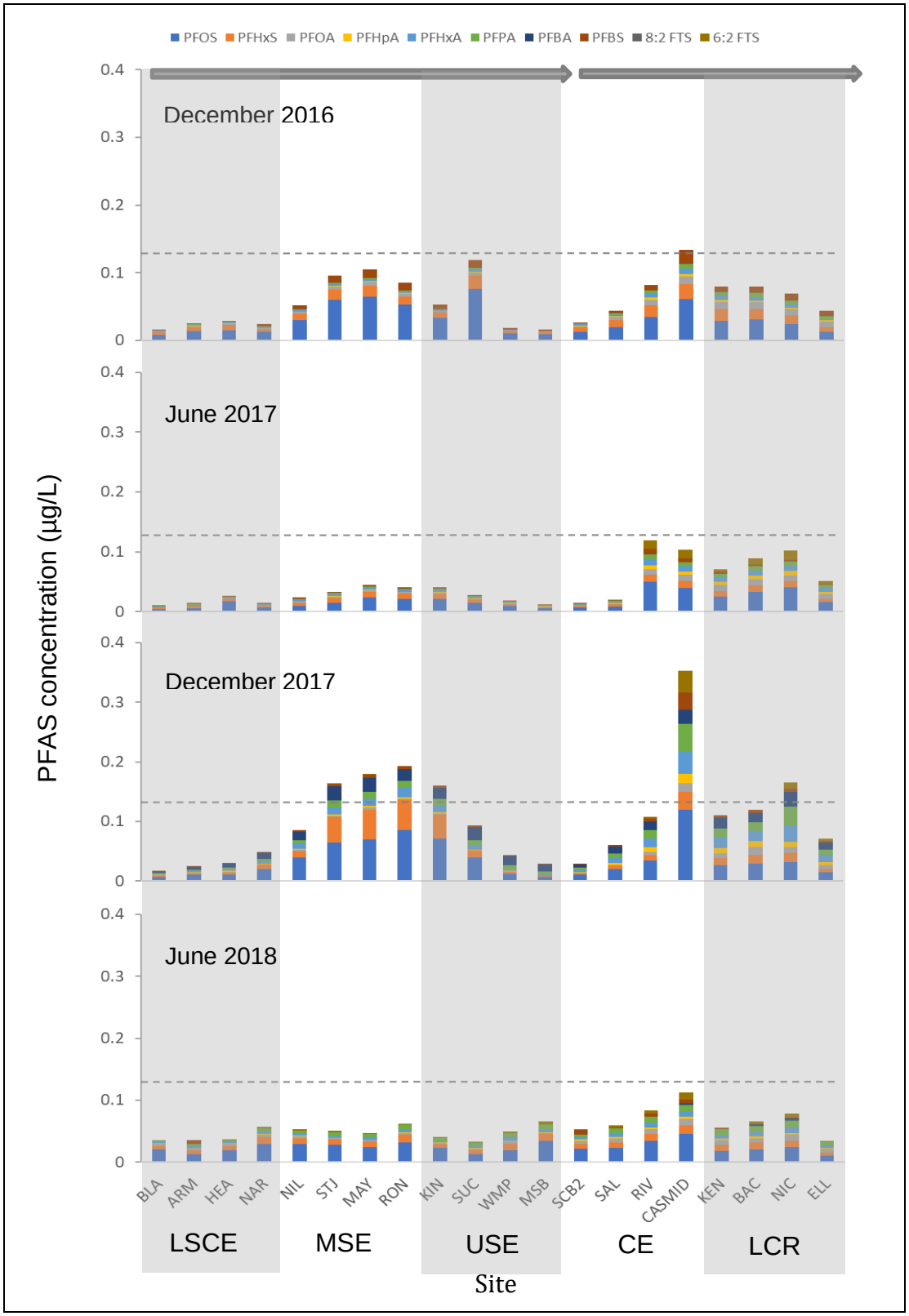

Figure 4. Total PFAS concentrations and contribution of different PFAS compounds at each site in the Swan Canning Estuary on each sampling occasion. The grey arrow at the top of the figure denotes an upstream gradient for the Swan Estuary (light grey) and the Canning Estuary (dark grey). The light grey shading demarcates the different estuary regions, from left to right; LSCE, MSE, USE, CE, and the LCR. The dashed line shows the draft ANZECC 95% species protection guideline.

Table 4. Summary table of key PFAS compounds in the Swan Canning Estuary ecological management zones

Date	Estuary Region	PFOS (µg/L) Mean	*SE*	PFHxS (µg/L) Mean	*SE*	PFOS+PFHxS (µg/L) Mean	*SE*	PFOA (µg/L) Mean	*SE*	∑PFAS (µg/L) Mean	*SE*	% PFOS+PFHxS Mean	*SE*
Dec-16	LSCE	0.012	*0.001*	0.006	*0.001*	0.018	*0.002*	0.002	*0.000*	0.023	*0.003*	75.3	*2.0*
	MSE	0.052	*0.008*	0.013	*0.002*	0.065	*0.009*	0.004	*0.001*	0.085	*0.012*	76.4	*0.3*
	USE	0.032	*0.016*	0.009	*0.004*	0.041	*0.019*	0.002	*0.001*	0.051	*0.024*	78.7	*0.8*
	CE	0.032	*0.011*	0.014	*0.003*	0.046	*0.014*	0.006	*0.002*	0.071	*0.024*	67.1	*3.0*
	LCR	0.024	*0.004*	0.013	*0.002*	0.037	*0.006*	0.010	*0.000*	0.068	*0.009*	53.2	*3.0*
Jun-17	LSCE	0.008	*0.003*	0.003	*0.001*	0.012	*0.003*	0.001	*0.000*	0.016	*0.004*	69.7	*4.1*
	MSE	0.017	*0.003*	0.008	*0.001*	0.025	*0.004*	0.002	*0.000*	0.035	*0.005*	70.1	*2.8*
	USE	0.012	*0.003*	0.005	*0.001*	0.017	*0.005*	0.002	*0.000*	0.025	*0.006*	67.3	*3.2*
	CE	0.026	*0.011*	0.007	*0.002*	0.033	*0.013*	0.006	*0.003*	0.064	*0.027*	54.8	*2.7*
	LCR	0.029	*0.005*	0.009	*0.001*	0.037	*0.007*	0.009	*0.000*	0.078	*0.011*	46.9	*2.5*
Dec-17	LSCE	0.012	*0.003*	0.004	*0.001*	0.016	*0.004*	0.000	*0.000*	0.031	*0.007*	51.8	*1.3*
	MSE	0.065	*0.010*	0.039	*0.009*	0.104	*0.019*	0.002	*0.001*	0.156	*0.024*	65.2	*2.7*
	USE	0.032	*0.015*	0.014	*0.009*	0.047	*0.023*	0.001	*0.001*	0.082	*0.030*	47.0	*9.0*
	CE	0.046	*0.025*	0.012	*0.006*	0.058	*0.031*	0.005	*0.004*	0.138	*0.073*	42.5	*0.9*
	LCR	0.025	*0.004*	0.012	*0.002*	0.037	*0.006*	0.009	*0.002*	0.118	*0.019*	31.6	*1.9*
Jun-18	LSCE	0.021	*0.004*	0.007	*0.001*	0.028	*0.005*	0.004	*0.000*	0.041	*0.005*	66.4	*4.6*
	MSE	0.029	*0.001*	0.010	*0.001*	0.039	*0.002*	0.002	*0.000*	0.054	*0.003*	72.2	*0.7*
	USE	0.023	*0.004*	0.009	*0.001*	0.031	*0.005*	0.002	*0.001*	0.047	*0.007*	64.8	*2.7*
	CE	0.031	*0.006*	0.011	*0.002*	0.042	*0.007*	0.007	*0.001*	0.078	*0.013*	54.1	*0.3*
	LCR	0.018	*0.003*	0.009	*0.001*	0.028	*0.004*	0.007	*0.001*	0.059	*0.009*	46.7	*1.7*

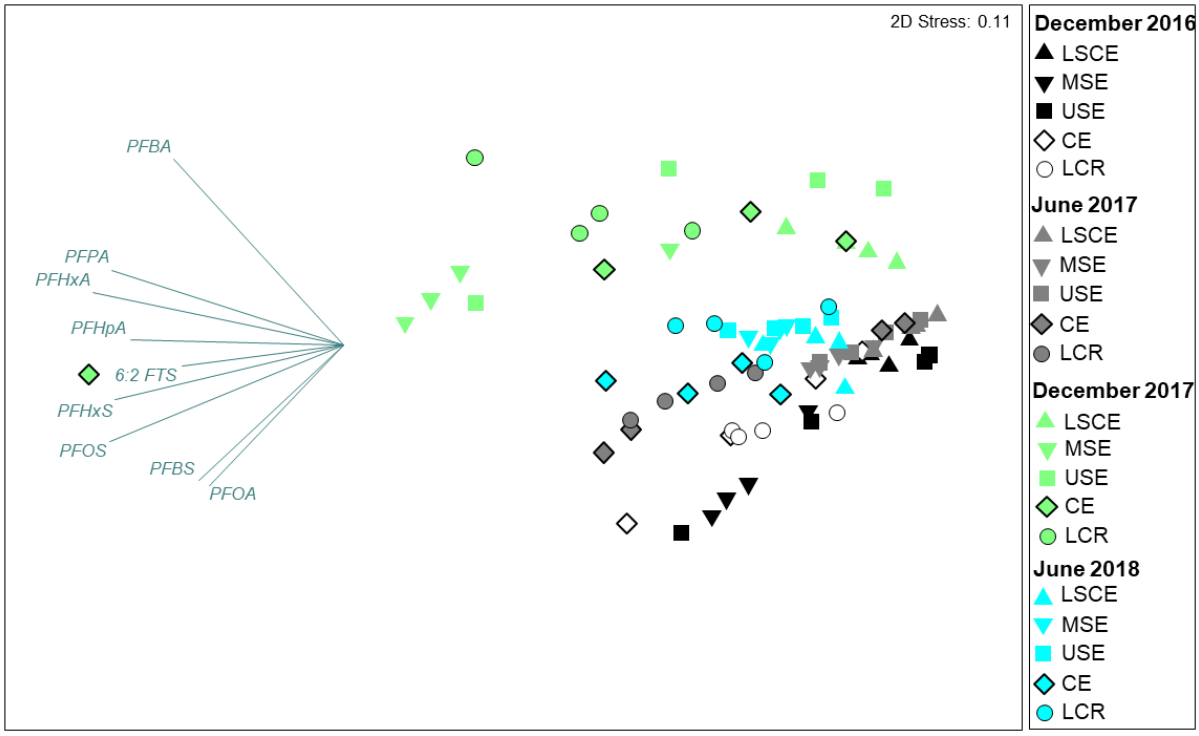

Figure 5. Non-metric multidimensional scaling ordination plot showing distances between samples based on the concentration of nine different PFAS compounds. The vectors demonstrate the directional influence of the compounds on the position of the data points.

Table 5. Mean water quality variables for each estuary ecological management zone and sampling occasion. DO (mg/L) = dissolved oxygen concentration, Temp = temperature.

Date	Estuary Region	Salinity		DO (mg/L)		pH		Temp (°C)	
		Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Dec-16	LSCE	29.08	*0.58*	7.04	*0.06*	7.93	*0.01*	23.5	*0.38*
	MSE	15.95	*2.43*	7.57	*0.28*	7.67	*0.07*	26.0	*0.13*
	USE	6.64	*0.48*	5.53	*0.89*	7.31	*0.06*	25.7	*0.36*
	CE	18.91	*4.84*	7.49	*0.81*	7.83	*0.10*	25.9	*0.77*
	LCR	0.54	*0.01*	7.59	*0.70*	7.63	*0.03*	25.8	*0.56*
Jun-17	LSCE	25.76	*0.17*	8.17	*0.40*	8.02	*0.04*	15.9	*0.11*
	MSE	21.03	*1.06*	10.81	*1.01*	8.02	*0.10*	15.5	*0.11*
	USE	14.51	*1.52*	8.95	*1.02*	7.47	*0.10*	14.7	*0.59*
	CE	19.25	*4.08*	6.73	*0.71*	7.70	*0.14*	15.8	*0.42*
	LCR	0.28	*0.04*	5.11	*0.38*	7.80	*0.05*	14.3	*0.27*
Dec-17	LSCE	30.16	*1.01*	7.22	*0.14*	8.06	*0.05*	23.5	*0.49*
	MSE	16.87	*2.19*	7.92	*0.07*	7.82	*0.06*	25.6	*0.10*
	USE	8.06	*0.58*	5.99	*0.70*	7.47	*0.05*	25.5	*0.17*
	CE	18.89	*5.23*	6.57	*0.47*	7.93	*0.14*	24.2	*0.25*
	LCR	0.55	*0.02*	6.22	*1.10*	7.59	*0.10*	23.3	*0.31*
Jun-18	LSCE	25.20	*3.17*	9.28	*0.18*	7.96	*0.02*	15.9	*0.31*
	MSE	8.07	*0.82*	8.73	*0.52*	7.69	*0.07*	15.3	*0.47*
	USE	4.76	*0.66*	8.27	*0.18*	7.60	*0.04*	13.7	*0.23*
	LCE	11.50	*4.90*	7.74	*0.97*	7.63	*0.17*	15.0	*0.73*
	LCR	0.32	*0.03*	6.54	*0.17*	7.91	*0.06*	14.2	*0.10*

5.2 Surface water – Catchment

Per- and polyfluoroalkyl (PFAS) substances were detected at every sampled catchment site on every sampling occasion (Figure 6 and 7). The draft PFOS AWQG for 99% species protection guideline was exceeded at every site except the Avon River site where it was below the laboratory detection limit on two occasions (December 2017 and June 2018) (Figure 6). Five sites (Mill Street Main Drain (MD), Ellen Brook Upstream (US) and Downstream (DS), Airport North MD and Airport South MD) recorded PFOS concentrations above the draft AWQG 95% species protection limit on at least one occasion and the Airport North MD exceeded the draft AWQG 90% species protection limit on three occasions. The recreational water quality guideline (for PFOS+PFHxS) was exceeded at the Airport North MD on all but one occasion (June 2018). The AWQG and the recreational water quality guideline for PFOA was not exceeded at any site.

No significant interaction effect in the concentration and composition of PFAS between EMZ and sampling period was observed (Pseudo $F_{9, 91} = 0.281$, $p = 1.0$), nor was there a significant difference between EMZs (Pseudo $F_{3, 91} = 1.686$, $p = 0.117$). However, significant variation was determined in the concentration and composition of PFAS between the sampling periods (Psuedo $F_{3, 91} = 5.249$, $p < 0.001$) and was significantly different ($p < 0.032$) across all time combinations except between December 2016 and June 2017 ($p = 0.189$). The highest concentrations of PFOS, PFHxS and total PFAS was in December 2017, consistently across all EMZ's (Table 6, Figure 6 and 7). At this time the percentage contribution to total PFAS from PFOS +PFHxS was the lowest recorded in each EMZ suggesting that other PFAS compounds may have contributed to the increase. The nMDS supported this assertion, in which the position of samples collected in December 2017 were impacted strongest by the short chain compounds PFBA and 6:2FTS (Figure 8). The percentage contribution of PFOS+PFHxS to total PFAS in the MSE was consistently among the highest of all EMZ's, ranging from 48.1% in December 2017 to 65% in December 2016 Table 9).

While no statistically significant differences between the EMZs were apparent, there were several individual sites where concentrations were consistently high. The highest concentrations of total PFAS, and particularly PFOS, were recorded in the Middle Swan Estuary catchment at Airport South MD (mean = 0.91 µg/L ± 0.22 SE PFAS and 0.44 µg/L ±0.10 SE PFOS) and in the Upper Swan Estuary catchment at Airport North MD (4.81 µg/L ± 1.29 SE PFAS and 2.79 µg/L ± 0.71 SE PFOS). The concentrations of total PFAS and PFOS at these two drains were greater than an order of magnitude higher than almost all other catchment sites (catchment global mean excluding Airport North and South MD total PFAS = 0.13 µg/L ± 0.017SE and PFOS = 0.039 µg/L ±0.005 SE). The percent contribution of PFOS+PFHxS to total PFAS ranged between 82% - 93%, far higher than the average of all other catchment sites (51 % (±3.3 SE)).

The spatial and temporal changes of PFAS in the Ellen Brook, a major tributary of the Swan River, are considerably noteworthy. Ellen Brook Downstream (Ellen Brook DS) flows year-round. In December 2016 and 2017, during summer baseflow in the

brook, total PFAS concentrations were 0.034 µg/L and 0.039 µg/L respectively (Figure 6). The contribution of PFOS and PFHxS to total PFAS was 49% in December 2016, and 28% in December 2017. However, during winter flows PFAS concentrations increased substantially to 0.082 µg/L in June 17 and 0.581 µg/L in June 2018 (Figure 6) and the contribution of PFOS+PFHxS increased to 72% and 74 % respectively. In June 2017 and 2018 the site located approximately 7 km upstream, Ellen Brook Upstream (Ellen Brook US) was flowing (and connected to Ellen Brook DS) and total PFAS concentrations of 0.249 µg/L in June 17 and 0.407 µg/L in June 2018 were recorded (Figure 6). The percentage contribution by PFOS+PFHxS was 88% and 72% respectively. Ellen Brook US was not flowing in December 2016 or December 2017.

In the Canning Estuary and Lower Canning River catchments, the highest total PFAS concentrations were consistently recorded at Mill Street MD, except in December 2017, when total PFAS concentrations at Bickley Brook (1.33 µg/L) were double that recorded at Mills St MD (0.577 µg/L) and approximately ten times higher than that recorded at all other times at that site (range 0.111 – 0.174 µg/L) (Figure 7). While PFOS concentrations remained consistent with that measured previously, the short chain compounds including PFPA, PFHxA and 6:2 FTS increased up to 44 times that previously measured (Figure 7). The PFOS concentrations at Mill Street MD were consistently the highest throughout the study (mean = 0.121 µg/L ± 0.025 SE).

Catchment water quality data indicated largely neutral pH and the range in temperature reflected the time of year, being at a minima in June and a maxima in December. Throughout the majority of sites conductivity was consistent with fresh water (Table 9). The high mean and large standard error in the Upper Swan Estuary drainage was reflective of the high conductivity at the Avon River site which flows saline throughout the year and in the Middle Estuary, the Maylands Main Drain site can be affected by the tidal brackish waters of the estuary.

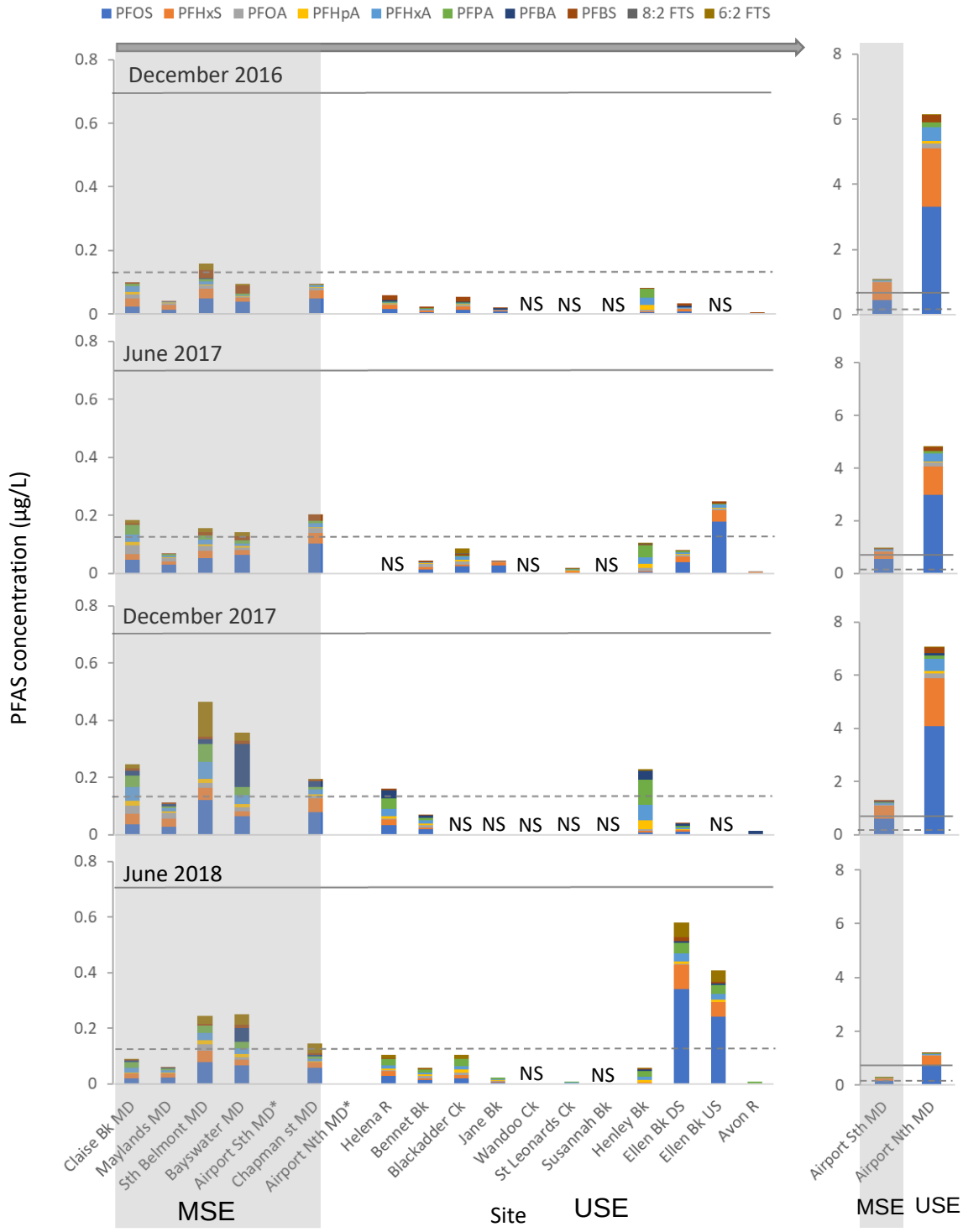

*Figure 6. Total PFAS concentrations and contribution of different PFAS compounds at each site draining to the MSE (shaded region) and USE (unshaded region) on each sampling occasion. The grey arrow at the top of the figure denotes the relative position of the confluence of each sampled drain sites along an upstream gradient in the Swan Estuary. *The Airport North and South Main Drains are plotted on a separate y-axis due to PFAS concentrations multiple orders of magnitude higher at those sites. The dashed and solid lines show the draft AWQG 95% species protection guideline for PFOS and the recreational water quality guidance value for PFOS and PFHxS, respectively. NS = not sampled*

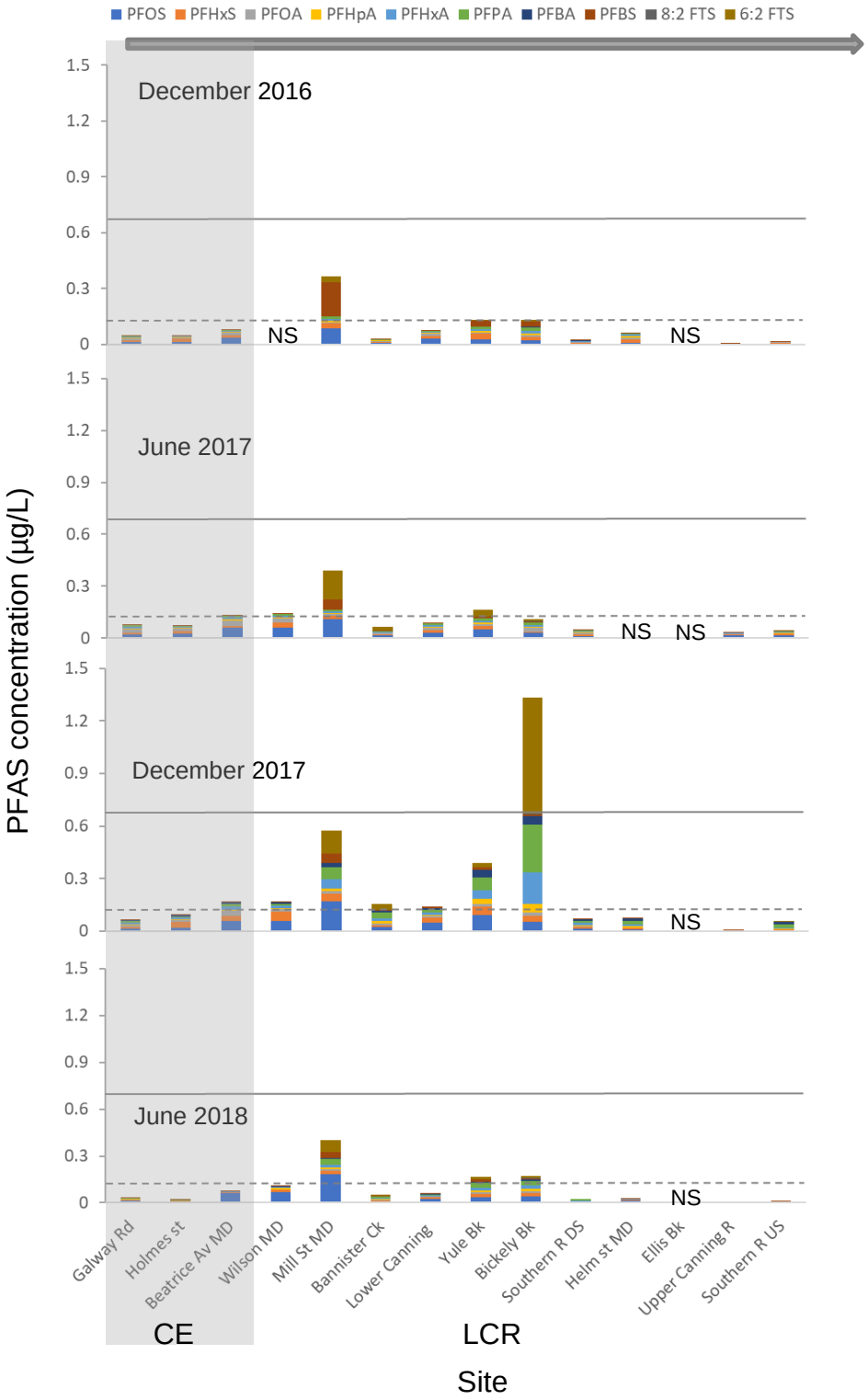

Figure 7. Total PFAS concentrations and contribution of different PFAS compounds at each site in the CE (shaded region), and the LCR (unshaded region) on each sampling occasion. The grey arrow at the top of the figure denotes the relative position of the confluence of each sampled drain sites along an upstream gradient in the Canning Estuary. The dashed and solid lines show the draft AWQG 95% species protection guideline for PFOS and the recreational water quality guidance value for PFOS and PFHxS, respectively. NS = not sampled

Table 6. Summary table of key PFAS compounds from the sub-catchments draining into each of the respective Swan Canning Estuary ecological management zones on each sampling occasion.

Date	Estuary Region	PFOS (µg/L)		PFHxS (µg/L)		PFOA (µg/L)		PFOS+PFHxS (µg/L)		∑PFAS (µg/L)		% PFOS+PFHxS	
		Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Dec-16	MSE	0.1040	*0.0700*	0.1104	*0.0880*	0.0081	*0.0016*	0.2144	*0.1579*	0.2631	*0.1656*	65.0	*7.0*
	USE	0.4199	*0.4114*	0.2302	*0.2243*	0.0221	*0.0199*	0.6502	*0.6357*	0.8035	*0.7637*	42.8	*8.7*
	CE	0.0306	*0.0146*	0.0163	*0.0038*	0.0073	*0.0017*	0.0469	*0.0180*	0.1149	*0.0633*	50.5	*6.4*
	LCR	0.0141	*0.0046*	0.0145	*0.0040*	0.0049	*0.0014*	0.0286	*0.0082*	0.0648	*0.0198*	45.8	*5.1*
Jun-17	MSE	0.1427	*0.0837*	0.0620	*0.0405*	0.0186	*0.0034*	0.2047	*0.1241*	0.2861	*0.1367*	59.4	*6.7*
	USE	0.3627	*0.3264*	0.1314	*0.1199*	0.0242	*0.0180*	0.4941	*0.4463*	0.6066	*0.5279*	61.4	*9.9*
	CE	0.0481	*0.0145*	0.0151	*0.0034*	0.0181	*0.0033*	0.0632	*0.0167*	0.1473	*0.0503*	45.4	*5.3*
	LCR	0.0250	*0.0056*	0.0099	*0.0025*	0.0119	*0.0033*	0.0350	*0.0080*	0.0811	*0.0201*	46.0	*4.3*
Dec-17	MSE	0.1548	*0.0900*	0.1107	*0.0760*	0.0188	*0.0030*	0.2655	*0.1657*	0.4448	*0.1777*	48.1	*9.6*
	USE	0.6954	*0.6809*	0.3056	*0.2989*	0.0299	*0.0261*	1.0010	*0.9798*	1.2642	*1.1620*	32.9	*12.2*
	CE	0.0575	*0.0239*	0.0286	*0.0074*	0.0145	*0.0032*	0.0861	*0.0288*	0.2039	*0.0755*	43.9	*6.7*
	LCR	0.0316	*0.0122*	0.0192	*0.0073*	0.0079	*0.0029*	0.0509	*0.0194*	0.2939	*0.1791*	32.5	*6.8*
Jun-18	MSE	0.0683	*0.0224*	0.0327	*0.0106*	0.0055	*0.0039*	0.1010	*0.0328*	0.1791	*0.0380*	54.1	*7.8*
	USE	0.1428	*0.0802*	0.0516	*0.0322*	0.0040	*0.0014*	0.1945	*0.1119*	0.2539	*0.1209*	44.0	*9.1*
	CE	0.0561	*0.0272*	0.0115	*0.0038*	0.0019	*0.0012*	0.0675	*0.0307*	0.1150	*0.0593*	61.8	*9.3*
	LCR	0.0170	*0.0063*	0.0096	*0.0033*	0.0040	*0.0021*	0.0266	*0.0096*	0.0665	*0.0278*	46.4	*5.9*

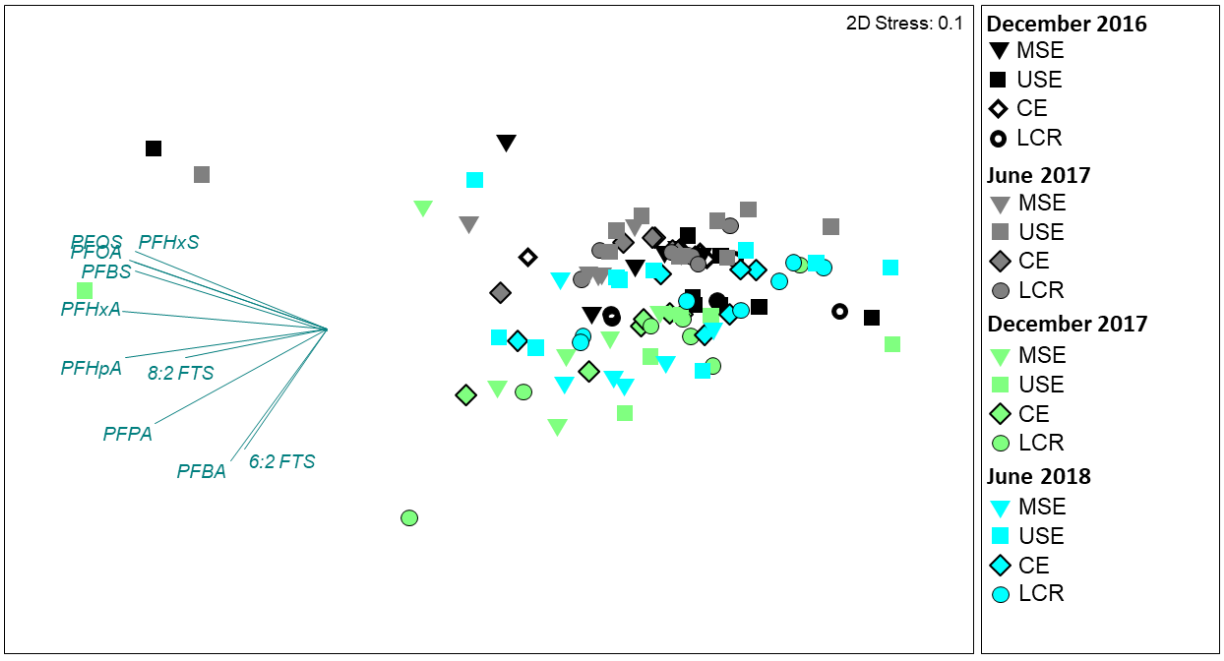

Figure 8. Non-metric multidimensional scaling ordination plot showing distances between samples based on the concentration of nine different PFAS compounds. The vectors demonstrate the directional influence of the compounds on the position of the data points.

Table 7. Mean water quality data for the catchment sites discharging to each Swan Canning Estuary ecological management zones on each sampling occasion. Cond (mS/cm) = conductivity, DO (mg/L) = dissolved oxygen concentration, Temp (°C) = temperature.

Date	Estuary region	Cond (mS/cm)		DO (mg/L)		pH		Temp (°C)	
		Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
Dec-16	MSE	0.76	*0.03*	7.01	*0.44*	7.48	*0.17*	23.0	*0.54*
	USE	2.37	*1.65*	4.75	*1.13*	7.41	*0.13*	22.1	*1.09*
	CE	0.69	*0.03*	3.81	*0.45*	7.18	*0.18*	20.5	*0.22*
	LCR	1.16	*0.23*	4.64	*0.44*	7.57	*0.07*	21.0	*0.52*
Jun-17	MSE	3.51	*2.82*	7.19	*0.30*	7.57	*0.17*	17.2	*0.50*
	USE	2.61	*1.82*	6.51	*0.84*	7.10	*0.21*	14.2	*0.36*
	CE	0.52	*0.07*	6.18	*1.05*	7.36	*0.24*	15.8	*0.50*
	LCR	0.55	*0.13*	5.53	*0.83*	7.41	*0.10*	15.0	*0.41*
Dec-17	MSE	0.72	*0.02*	7.59	*0.79*	7.46	*0.13*	24.6	*0.80*
	USE	3.28	*3.00*	5.41	*1.07*	7.34	*0.13*	24.9	*1.14*
	CE	0.64	*0.04*	4.93	*0.54*	6.89	*0.11*	21.3	*0.33*
	LCR	0.84	*0.15*	4.35	*0.64*	7.42	*0.05*	22.3	*0.46*
Jun-18	MSE	0.66	*0.09*	7.77	*0.72*	7.44	*0.05*	17.6	*0.95*
	USE	1.96	*1.11*	7.56	*0.51*	7.40	*0.06*	14.8	*0.50*
	CE	0.37	*0.04*	7.26	*0.55*	7.24	*0.21*	16.6	*0.61*
	LCR	0.54	*0.07*	7.43	*0.76*	7.64	*0.09*	14.7	*0.54*

5.2.1 PFAS load and rainfall

Estimated PFAS loads in December were generally low in comparison to June (Figure 9). The highest load was recorded at Bayswater MD in both December 2016 and 2017 (Figure 9). However, this calculation was based on historical flows and may not have reflected the conditions at the time. The estimated loads in Mill Street MD were the highest of the drains with current stream gauge data. Estimated total PFAS and PFOS+PFHxS loads were marginally higher in 2017 than 2016.

The estimated load of total PFAS and PFOS+PFHxS in June ranged from two to ten times higher than December (Figure 9). Estimated loads at Mill Street MD were the highest in June 2017. The estimated total PFAS load of 695 mg/hr, was the highest in this study. Ellen Brook US had the highest estimated total PFAS load in June 2018 and the estimated PFOS+PFHxS load of 371 mg/hr was the highest in the study. Ellen Brook US did not flow during December 2016 or 2017. The drains that consistently contributed the highest loads were Mill Street MD, South Belmont MD, and Yule Brook (Figure 9). PFAS loads in Bayswater MD in summer were high but given the lack of accurate stream gauging at this site, this result is indicative only. The Yule Brook gauging station was not operating in June 2018 and thus load was calculated using historical discharge data. Please note that the drains where the highest concentrations were detected, Airport North and Airport South Main Drains, do not have stream gauging stations and could not be included in this analysis despite a high potential for large PFAS loads from these drains.

Total yearly rainfall (at the Bureau of Mereology Perth Airport station) over the study period varied considerably from 674.4 mm in 2016 to 743.8 mm in 2018 (Figure 10).

The four-week period preceding the June 2018 sampling was the wettest pre-sampling period in the study with 150.2 mm recorded compared to 47.4 mm prior to June 2017, 7.4 mm prior to December 2017 and 5.6 mm prior to December 2016 sampling. It should be noted that rainfall does vary throughout the catchment.

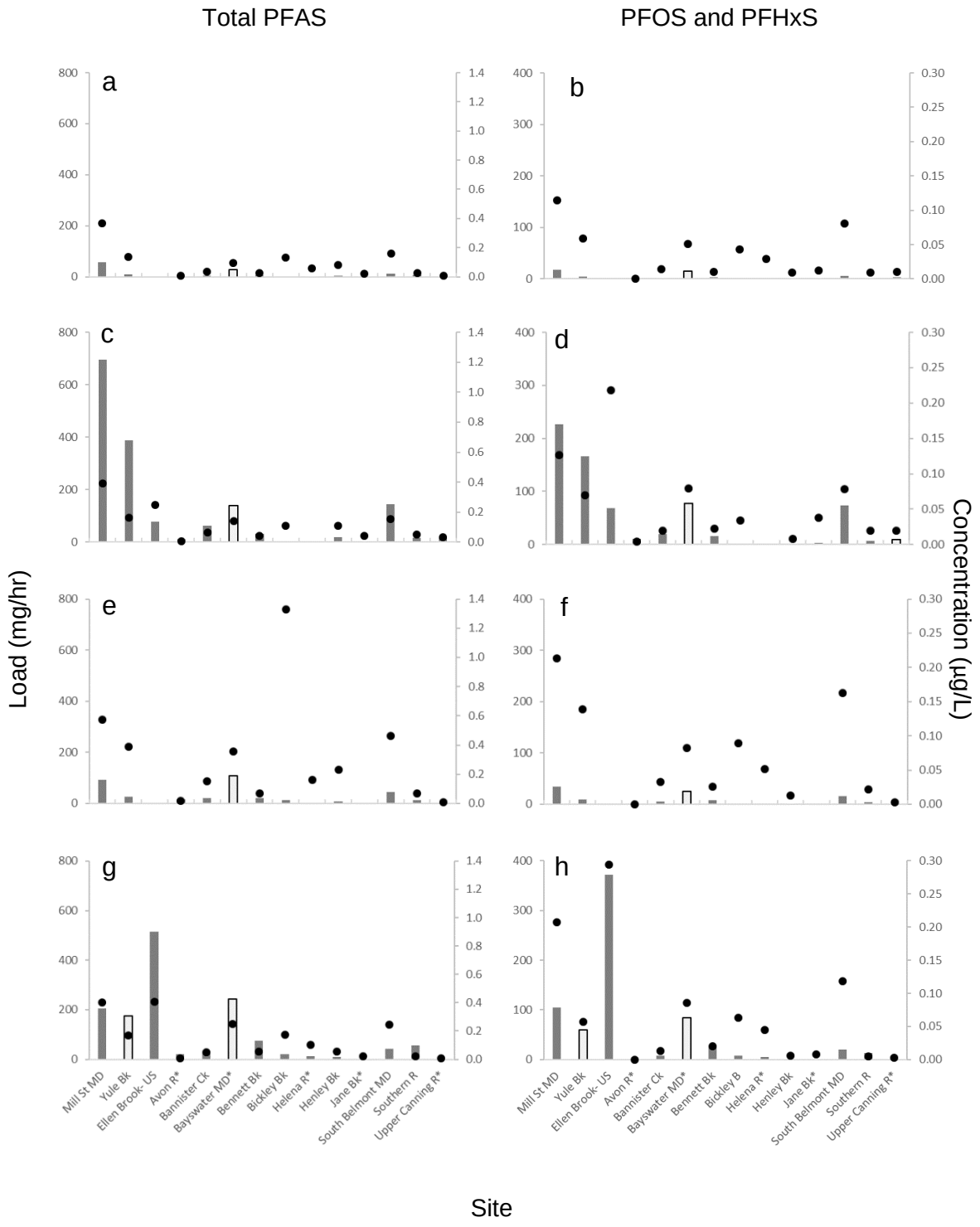

*Figure 9. Total PFAS and PFOS+PFHxS loads and concentrations at sites with nearby gauging station with a, b) December 2016, c and d) June 2017, e and f) December 2017 and, g and h) June 2018. Bars represent the estimated load (left axis) and the solid circles represent the concentration (right axis). Sites using historical discharge are indicated by the * and light grey vertical bars.*

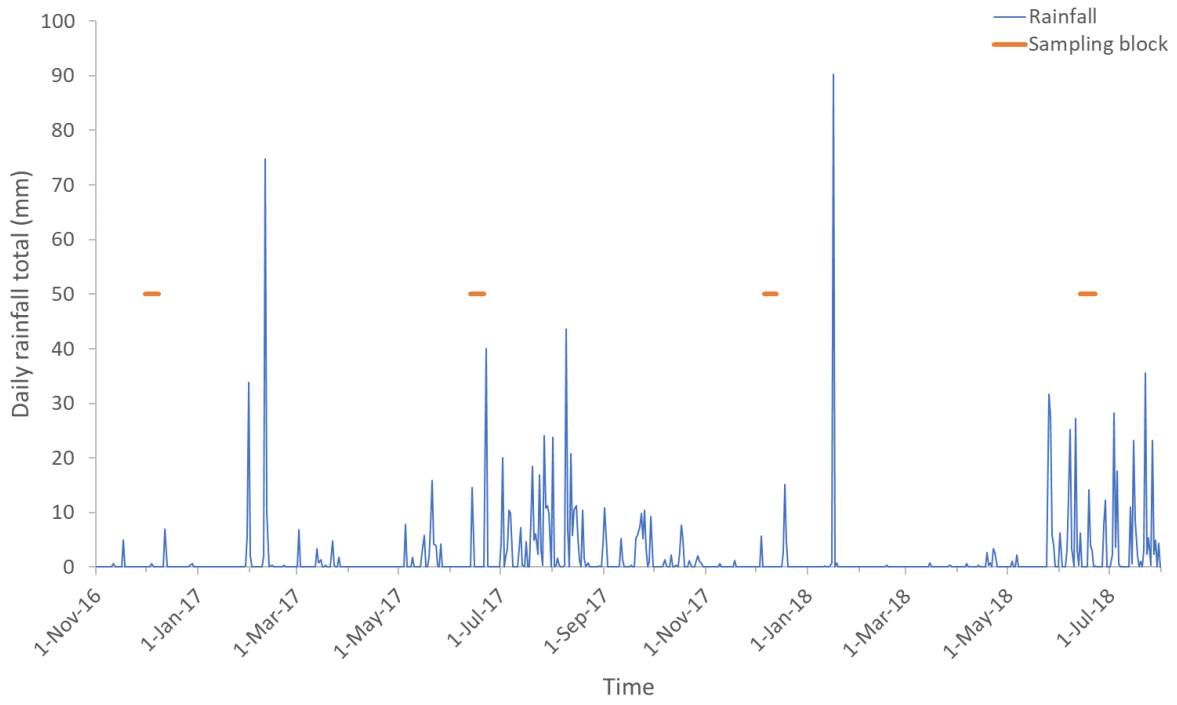

Figure 10. Rainfall recorded at the Australian Bureau of Meteorology weather station at Perth Airport over the study period. Sampling time periods are shown by the thick orange bar.

5.3 Biota

5.3.1 Black bream

In the three tissue types analysed, PFOS and PFHxS were the dominant compounds (Figure 11). These compounds were the only two detected in the muscle tissue and comprised 98% of the mean liver concentration and 95% of the mean carcass concentration. In muscle tissue, PFOS was detected in 29 of the 30 samples (Figure 11a), had a mean concentration of 0.0023 mg/kg (±0.0004 SE) (Figure 11b) and constituted 99.5% (±0.38% SE) of the total PFAS concentration. Only two muscle samples returned a detection of PFHxS which contributed 0.5% (±0.38% SE) of the total PFAS concentration. In black bream liver samples, seven of the 14 compounds were detected, three of which, PFOS, PFHxS and PFOA, were detected in multiple samples, whilst the remaining four compounds were single detections (Figure 11a). The mean concentration of PFOS in the liver was 0.0235 mg/kg (±0.004 SE) and it constituted 89.4% (±2.55% SE) of the total PFAS concentration (Figure 10b). Six PFAS compounds were detected in the fish carcass; PFOA, PFNA, PFDA, PFDoA, PFHxS and PFOS. PFOS was detected in every sample, had a mean concentration of 0.0102 mg/kg (±0.0018 SE) and comprised 90.7% (±2.24% SE) of the total PFAS concentration (Figure 11b).

The compounds that were detected in the body tissues were dominated by the longer chain perfluoroalkyl sulfonates (PFHxS and PFOS) while some long chain carboxylic acids were also detected (PFHpA, PFOA, PFNA, PFDA and PFDoDA)

(Figure 11a). There were no detections of PFBuA, PFPeA, PFHxA, PFHpA and PFBS or the fluorotelomers 6:2 and 8:2 FTS.

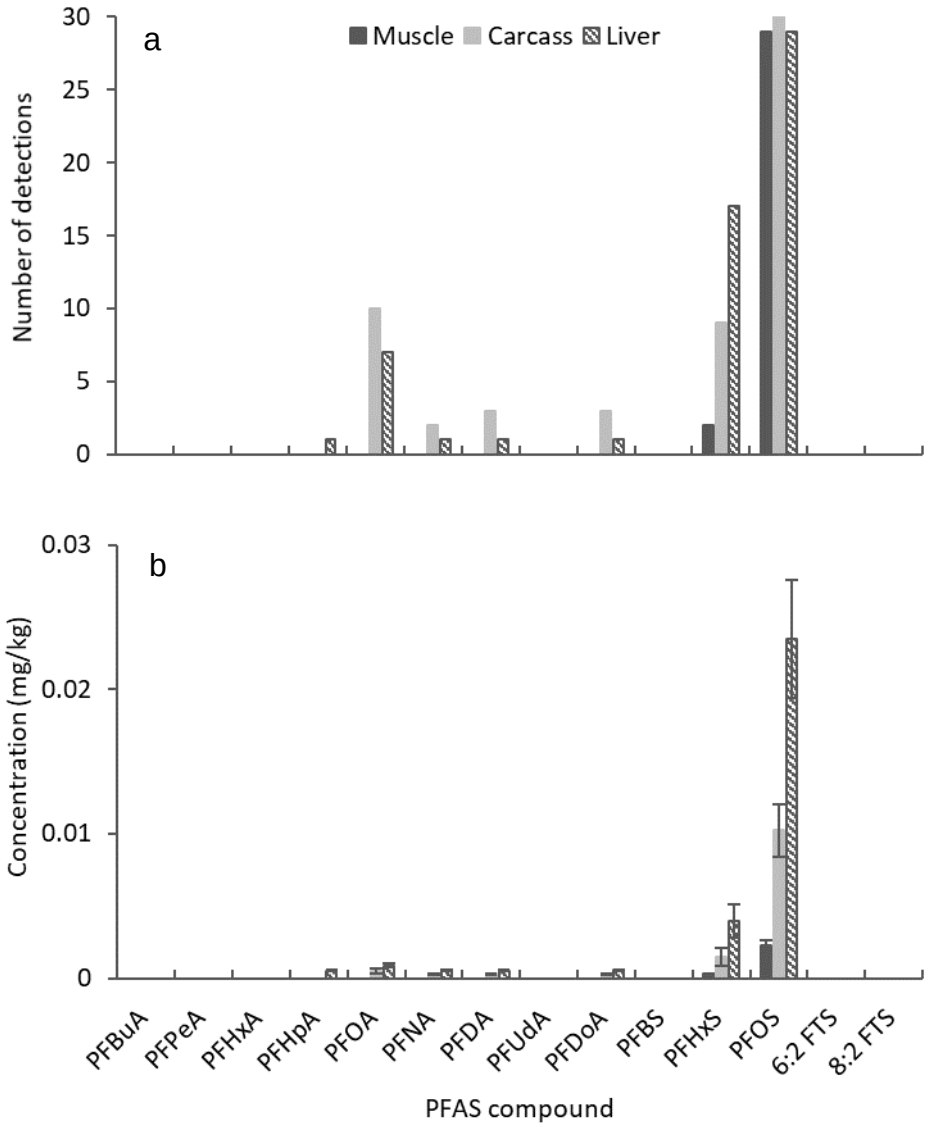

Figure 11. a) The frequency of detections (n = 30) of PFAS compounds in three types of body tissue (muscle, carcass and liver) of A. butcheri, *and b) the mean concentration of PFAS compounds in the same body tissues. Along the x axis carboxylic acids are presented first, followed by sulphonic acids and finally the fluorotelomers.*

The comparison of PFOS+PFHxS concentration between sex, region of capture (EMZ), and tissue type demonstrated a significant two-way interaction between sex and region only ($F_{1, 78} = 9.413$, $p = 0.003$), suggesting that the effect of sex on PFAS

concentrations differed between the regions. This was evident in the PFAS tissue concentrations in male fish which were consistent between the regions, while the concentrations in female fish, in all tissues were higher in the Swan Estuary than the Canning Estuary (Figure 12). There was no significant interaction between tissue type or region ($F_{2, 78} = 0.572$, $p = 0.567$), however the tissue concentrations were significantly different between tissues (main effect: $F_{2, 78} = 41.139$, $p < 0.001$), with tissue concentration highest in the liver > carcass > muscle.

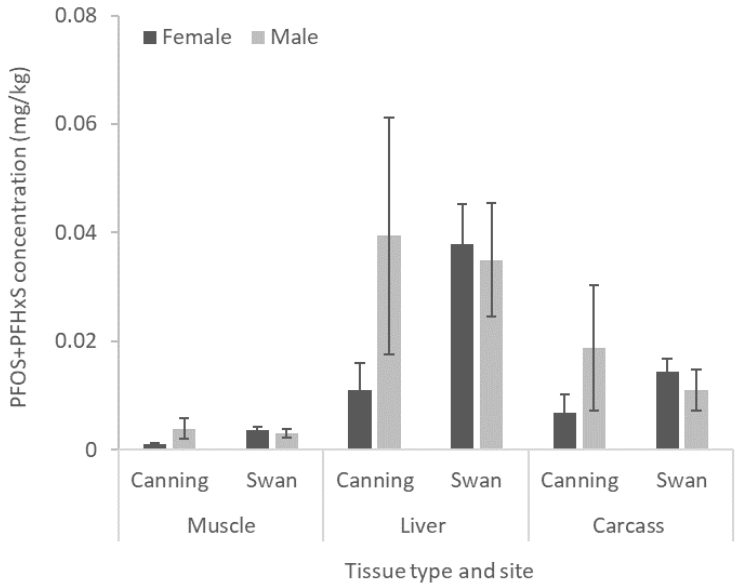

Figure 12. Comparison of the mean A. butcheri *female and male tissue PFOS+PFHxS concentration in the Canning Estuary (Canning) and Middle Swan Estuary (Swan).*

Black bream - PFAS body burden and accumulation

The estimated whole fish concentration of PFOS+PFHxS was not affected by the length of the male or female fish (male - $R^2 = 0.034$, $p = 0.563$; female - $R^2 = 0.055$, $p = 0.345$) (Figure 13a). Furthermore, there was no significant relationship between whole fish PFOS+PFHxS concentration and male or female GSI (male - $R^2 = 0.037$, $p = 0.548$; female - $R^2 = 0.113$, $p = 0.172$) or HSI (male - $R^2 = 0.010$, $p = 0.755$; female - $R^2 = 0.001$, $p = 0.871$) in this study (Figure 13b and 13c, respectively).

The mean PFOS+PFHxS body burden of all 30 fish was 2.95 µg (±0.65 µg SE). The body burden of female fish was 2.71 µg (± 0.89 µg SE) (TL range 245 mm – 340 mm) and male fish was 3.31 µg (± 0.97 µg SE) (TL range = 248 mm - 345 mm). The highest body burden of a fish caught in this study was 16.56 µg PFOS+PFHxS in a female fish (TL = 340 mm, weight = 603 g) caught in the Canning Estuary. There was a significant positive relationship between PFOS+PFHxS burden in female fish and total length ($R^2 = 0.220$, $p = 0.049$), however the PFOS+PFHxS burden in male fish was not affected by fish length ($R^2 = 0.005$, $p = 0.815$) (Figure 13d).

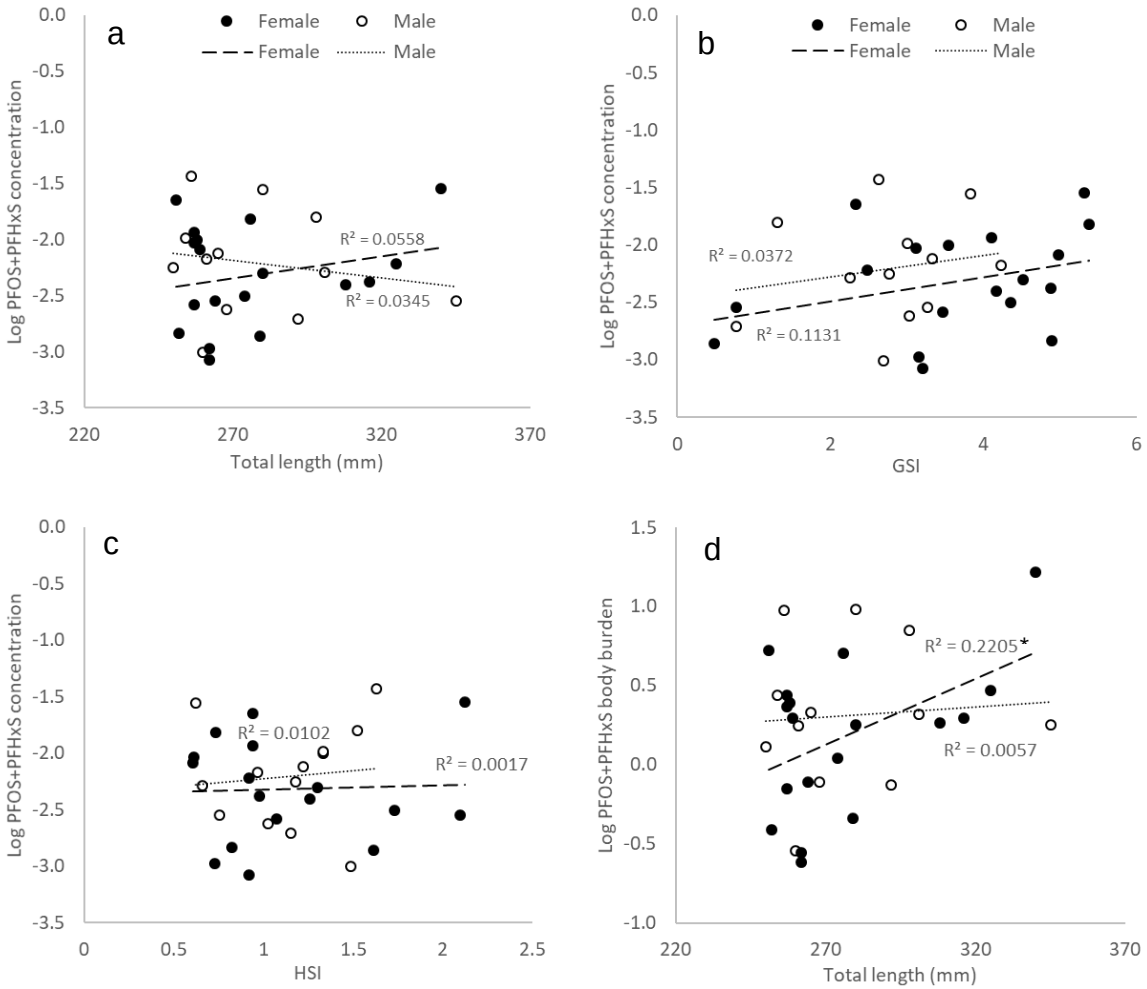

*Figure 13. The relationship between a) calculated whole fish PFOS+PFHxS concentration and total length, b) whole fish concentration and gonadosomatic index, c) whole fish concentration and hepatosomatic index and d) PFOS+PFHxS body burden and total length. * denotes a significant regression line.*

Black bream- Tissue partitioning

Four tissues types (muscle, liver, carcass and gonads) in three male and three female fish from the Middle Swan Estuary were analysed to examine tissue partitioning of PFOS+PFHxS. The concentration of PFOS+PFHxS in the body tissues varied significantly between tissue type and sex (two-way interaction = $F_{3, 16} = 3.577$, P = 0.038), with the difference likely attributable to the much higher concentration of PFOS+PFHxS in the gonads of female fish (PFOS+PFHxS = 0.075 mg/kg ± 0.017SE) than male fish (0.006 mg/kg ± 0.002SE) (Figure 14a). The PFAS concentration ranking, lowest to highest, in the different tissues for each sex is given below:

Male = muscle < gonad < carcass < liver

and

Female = muscle < carcass < liver < gonad

Of the six fish analysed for this component of the study, the total body burden of female fish was 3.40 µg (±0.95 µg SE) and 1.87 µg (±0.58 µg SE) for male fish. The tissue type with the largest mass, the carcass, was the greatest contributor to whole fish PFOS+PFHxS body burden, contributing 72% and 78% female and male, respectively. The contribution of the carcass, muscle and liver to the total body burden was reasonably consistent between the male and female fish (Figure 14b). The contribution of PFOS+PFHxS load in the gonads differed substantially and was far greater in the females (15%) than the males (5%), consistent with the result for tissue concentrations.

The contribution of each of the major tissues sampled to total PFAS body burden was different and varied according to sex (Figure 14b):

Male = liver < gonad < muscle < carcass

and

Female = liver < muscle < gonad < carcass

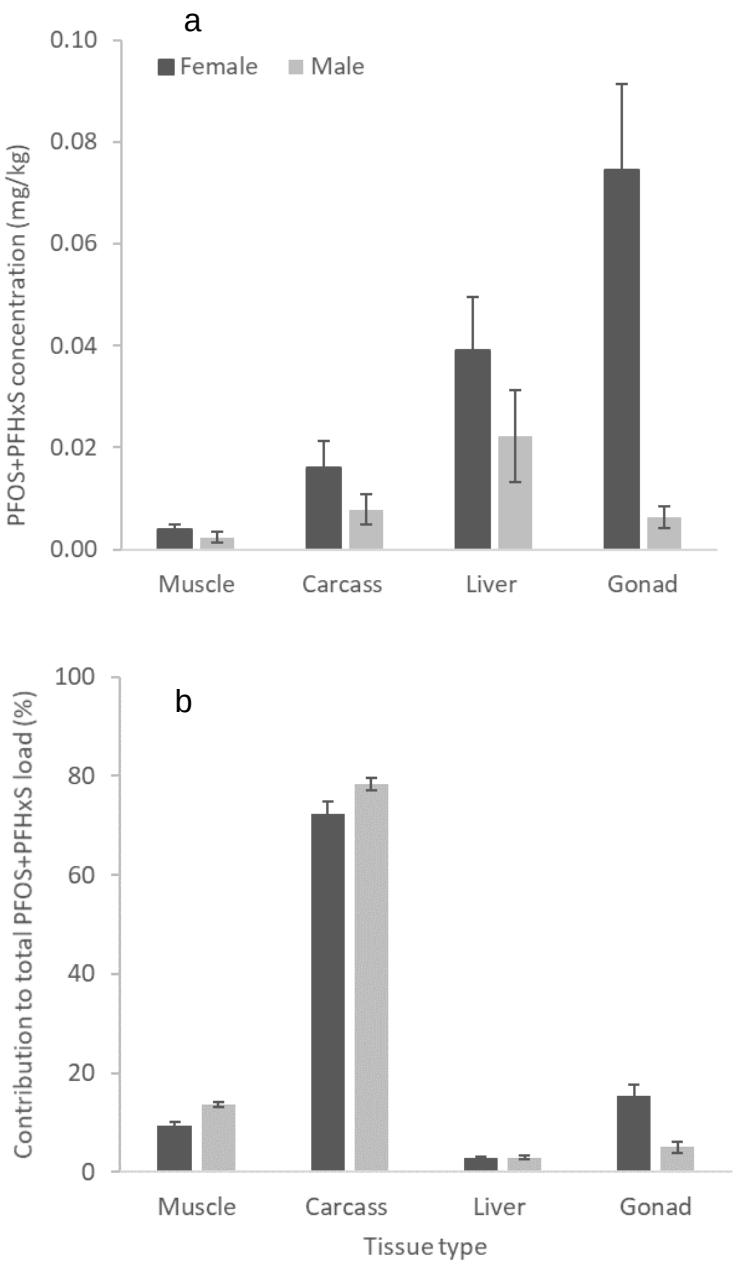

Figure 14. The a) combined concentration of PFOS and PFHxS in muscle, liver, gonad and carcass tissue and b) contribution of each tissue to the total PFOS and PFHxS burden in female and male A. butcheri *from the Middle Swan Estuary.*

5.3.2 Blue swimmer crabs

Eight PFAS compounds were detected in the muscle and seven were detected in the viscera of blue swimmer crabs (Figure 15a). The dominant compound detected in the different crab tissues was PFOS, averaging 57.8% (±2.63% SE) in the muscle and 80.8% (±1.63% SE) of the total PFAS concentration in the viscera. In the muscle tissue, PFHxS averaged 16.7% (±1.78% SE) of the total concentration and PFOA averaged 13.7% (±1.45% SE) (Figure 15b). There were a greater number of detects of PFHpA, PFOA, PFDA, PFUdA and PFDoA in muscle than viscera tissue despite

lower mean concentrations (Figure 15a, b). It is worth noting however, that muscle and viscera tissue had different laboratory limits of reporting and, as such, low concentrations of some compounds may not have been detected in the viscera.

The compounds that were detected in the body tissues were dominated by the longer chain perfluoro sulfonates (PFHxS and PFOS) while some long chain carboxylic acids were also detected (PFHpA, PFOA, PFNA, PFDA, PFUdA, and PFDoDA) (Figure 15a). There were no detections of PFBuA, PFPeA, PFHxA, PFBS, or the fluorotelomers 6:2 and 8:2 FTS in blue swimmer crabs during this study.

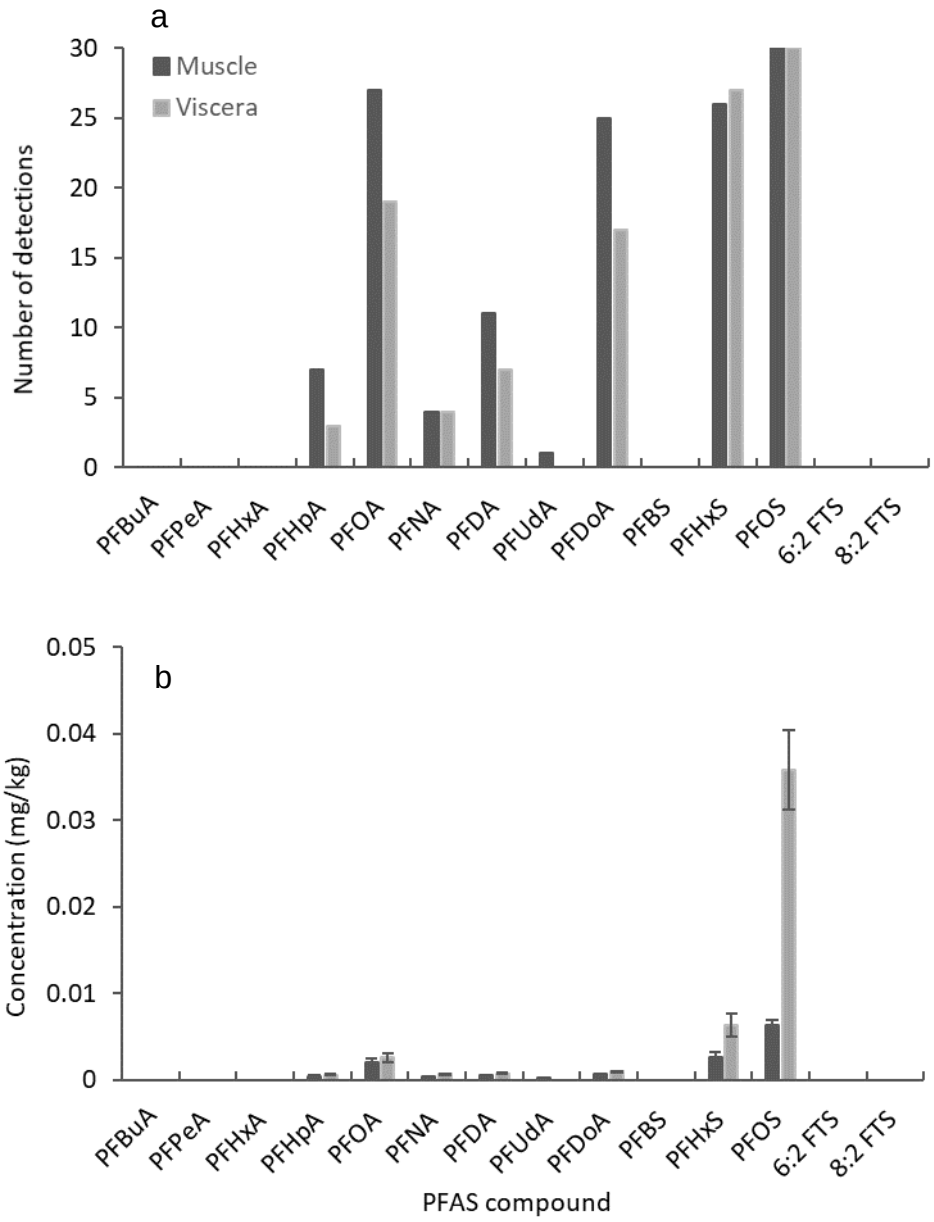

Figure 15. a) The number of detections of PFAS compounds in two types of body tissue (muscle and viscera) of P. armatus *and b) the mean concentration of PFAS compounds in the same body tissues.*

PFAS body burden and accumulation

There was no significant interaction effect in PFAS concentrations between sex and tissue type ($F_{1, 56}$ = 1.955, p = 0.168) (Figure 16). The concentration of PFOS+PFHxS in female crabs was higher than males in both the viscera and the muscle tissue ($F_{1, 56}$ = 13.48, p = 0.001). The mean concentration of PFOS+PFHxS in the viscera (0.042 mg/kg ±0.006 SE) was significantly higher than muscle tissue (0.009 mg/kg ±0.001 SE) ($F_{1, 56}$ = 120.78, p <0.001).

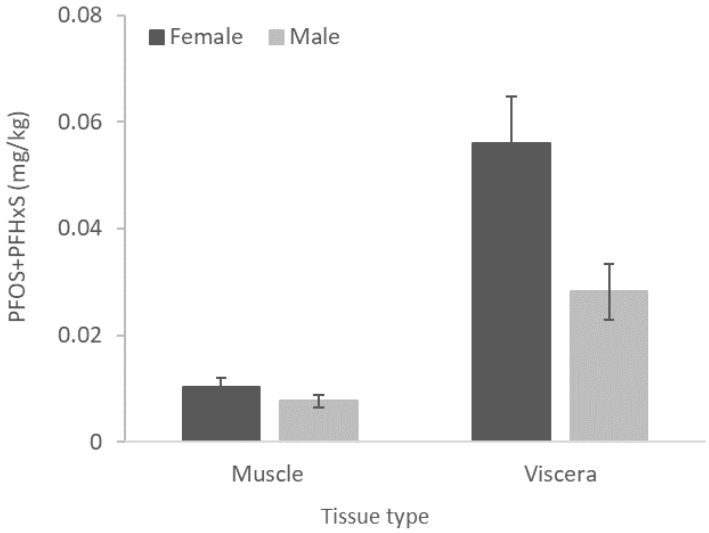

Figure 16. The mean concentration of PFOS+PFHxS in male and female muscle and viscera tissue.

There was no relationship between carapace width of the crab and the muscle or viscera PFOS+PFHxS concentration in either male (R^2 = 0.018, p = 0.627, and R^2 = 0.052, p = 0.411 respectively) or female crabs (R^2 = 0.000, p = 0.967, and R^2 = 0.006, p = 0.777 respectively) (Figure 17a and b). The relationship between PFOS+PFHxS burden and carapace width was also not significant in female muscle (R^2 = 0.070, p = 0.334), viscera (R^2 = 0.016, p = 0.645) and combined (R^2 = 0.021, p = 0.604) tissue burden, and male muscle burden (R^2 = 0.195, p = 0.099) (Figure 17c, d and e). However, a significant positive relationship was evident between carapace width and both male PFOS+PFHxS burden in viscera (R^2 = 0.269, p = 0.047) and total burden (R^2 = 0.264, p = 0.050) (Figure 17d and e).

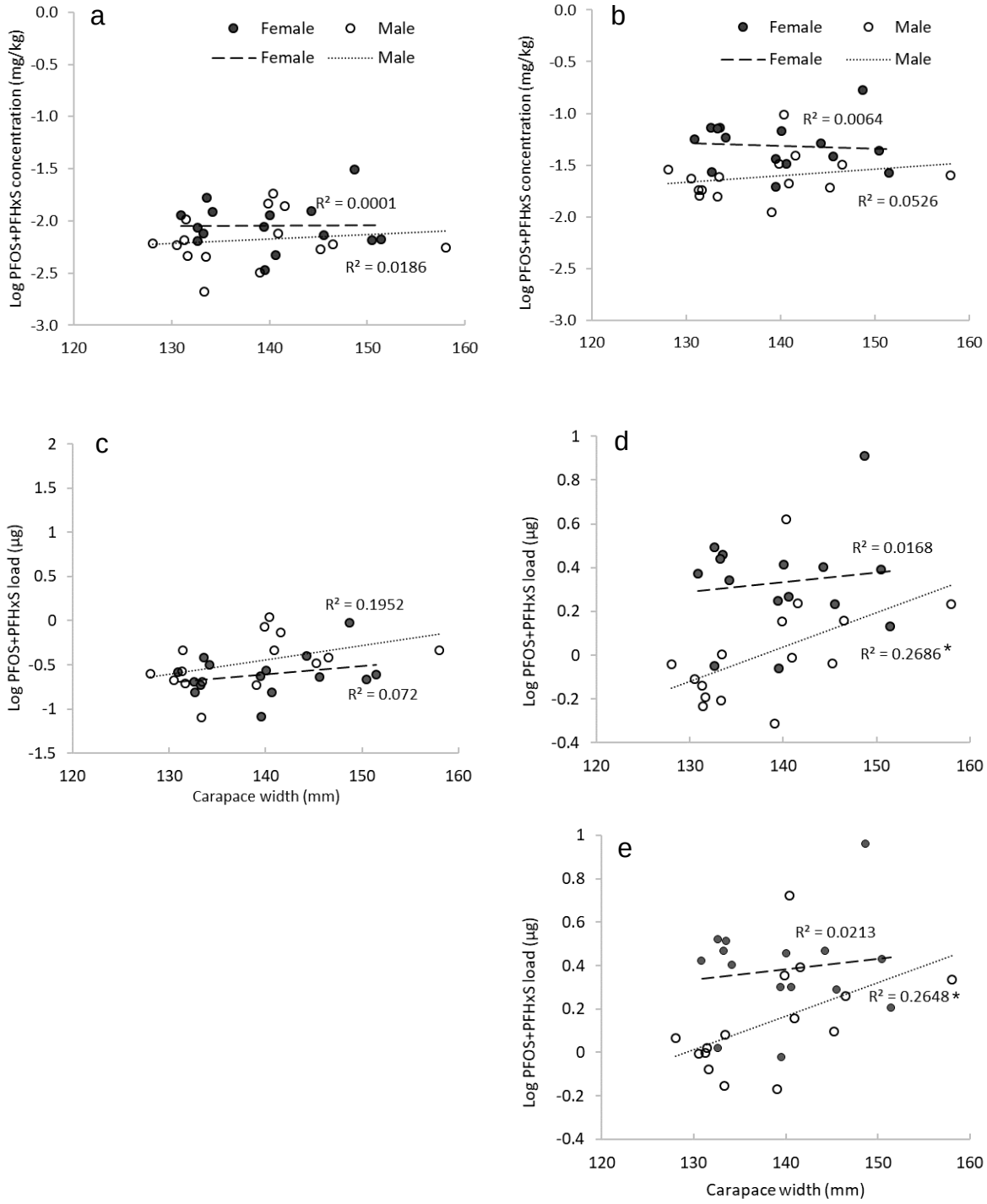

*Figure 17. The relationship between PFOS+PFHxS concentration and carapace width in a) muscle tissue, b) viscera tissues, and PFOS+PFHxS load and carapace width in, c) muscle tissue, d) viscera tissue, and e) the sum of muscle and viscera tissue. The regression lines are dashed= females and fine dots = males. * denotes a significant regression line.*

5.3.3 Consumption guidance

The mean PFOS+PFHxS concentration in the edible portion (muscle) of both black bream and blue swimmer crabs resulted in a CR_{mm} 126 and 70 respectively (Table 8). This means that in order to be at risk of adverse effects from PFOS+PFHxS exposure from the consumption of the targeted seafood, a person would need to consume more than 126 and 70 meals per month of black bream and blue swimmer crab respectively over their lifetime. The benchmark used in this study from which a restriction of consumption should be recommended is <16 meals per month (Hoeksema 2015, USEPA 2000). Thus, the mean PFOS+PFHxS and PFOA concentration in the black bream and blue swimmer crab muscle was not high enough to advise a restriction in consumption (Table 8). It must be noted that this advice does not account for the potential antagonistic, synergistic or additive effects of multiple contaminants in the fish or crab and does not account for exposure from other sources, including other dietary sources.

*Table 8. Consumption guidance for black bream (*A. butcheri*) and blue swimmer crab (*P. armatus*) muscle, from the Swan Canning Estuary.*

		Fish muscle		Crab muscle	
		PFOS+PFHxS	PFOA	PFOS+PFHxS	PFOA
TDI	(µg/kg-bw/d)	0.02	0.16	0.02	0.16
C_m	(µg/kg)	2.53	ND	9.05	2.01
C_{SE}	(±µg/kg)	0.401		1.05	0.417
CR_{lim}	(kg/d)	0.621		0.174	6.24
CR_{mm}	**meals/month**	**126.11**		**70.43**	**2531.12**

TDI = Tolerable Daily Intake

C_m = average concentration of contaminant; **C_{SE}** = standard error of C_m

CR_{lim} = Daily Consumption Limit; **CR_{mm}** = Average Meal Consumption Limit

6 Discussion

6.1 Surface water

Per- and polyfluorinated alkyl substances (PFAS) were detected throughout the Swan Canning Estuary and its catchment. The draft PFOS AWQG for 99% species protection (0.00023 µg/L PFOS) was exceeded at every site throughout the estuary and catchment, excluding the Avon River site. At the Avon River site, upstream of any major urban and industrial influences, PFOS, PFHxS, and PFOA were not detected on two occasions (December 2017 and June 2018), suggesting that the background concentration of these compounds within the catchment of the Swan Estuary was below the current laboratory detection limit (0.0003 µg/L) and thus potentially below the draft AWQG 99% species protection limit. The draft 95% species protection guideline (0.13 µg/L PFOS) was not exceeded at any estuary site, but was exceeded at five catchment sites; Perth Airport North and South Main Drains

on each sampling occasion, Mill Street Main Drain in December 2017, Ellen Brook Upstream in June 2017 and 2018 and Ellen Brook Downstream in June 2018. The draft 90% species protection limit (2.0 µg/L PFOS) was exceeded at Airport North Main Drain on three of the four sampling occasions (excluding June 2018).

In the catchment, the highest concentrations of PFAS were consistently observed in the Airport Main Drains (draining into the USE and MSE) and Mill Street Main Drain (CE). When Ellen Brook US (USE) was flowing elevated concentrations were also observed at this site. These four sub-catchments likely represent the major surface water sources of PFAS into the estuary. The composition of total PFAS (summed) in the Swan Canning Estuary catchment was highly varied, however, PFOS and PFHxS were dominant compounds in Airport South and Airport North Main Drains, and the Ellen Brook (in June). The contribution of PFOS and PFHxS to total PFAS in these three catchments was typically above 70% (when Ellen Brook US was flowing), far higher than the average of all catchments (51%) indicating a high likelihood of AFFF source. The catchment areas of these three drains all contain an airport (Perth Airport - Perth Airport North and South Main Drain) or airbase (Pearce Airbase - Ellen Brook) where PFAS contaminated soil and water has been identified, primarily consisting of PFOS and PFHxS (Ascot 2018, GHD 2018). The historical use of firefighting foams within these sites is acknowledged as the major source of contamination and the results in the current study indicate that it is migrating off site in surface water. Interestingly, the increase in PFAS concentration and the change in composition (PFOS and PFHxS became the dominant compounds) that occurred at the Ellen Brook DS site when flow was recorded at Ellen Brook US suggested that during winter flows the upper catchment draining Pearce Airbase was connected to the lower catchment and consequently the estuary. This impact was observed though the increase in PFAS concentration in the USE in June 2018. The other drainages varied in the PFAS concentration and composition, although PFOS and PFHxS were present at all sites, except the Avon River site.

Within the estuary the total PFAS concentration was consistently highest in the MSE, CE and the LCR, largely reflecting the concentrations in those key sub-catchments discharging to each EMZ. In addition, much lower concentrations of PFAS at the most upstream sites within the LCR (Ellison Parade) and the USE (West Midland Pool and Middle Swan Bridge), well outside the influence of most known contaminated drain and stream discharge points, provided further evidence that the major sources of PFAS contamination to the estuary were within the middle estuary regions. The concentrations of PFAS were lowest in the Lower Swan Canning Estuary (LSCE) on all sampling occasions from a result of increased mixing and dilution as the estuary greatly expands in size and volume in this region relative to other EMZs, coupled with greater tidal exchange with the ocean. There is some evidence in the literature to suggest that the solubility of some PFAS compounds declines in saltwater, for example increasing salinity under specific circumstances, such as the presence of calcium and manganese ions in the water column can greatly decrease water solubility and enhance sediment adsorption (Chen et al. 2012, Zareitalabad et al. 2013, Ololade et al. 2016). In the Swan Canning Estuary, salinity increased along a downstream gradient and is most saline (and close to

marine salinity) in the LSCE for much of the year. The combination of increased dilution and decreasing solubility of PFAS may have had an impact on the prevalence of PFAS in the lower estuary. While the concentration of total PFAS was lowest in the LSCE the composition of total PFAS was dominated by PFOS and PFHxS consistent with the MSE and USE. However, in the Canning Estuary short chain PFAS, PFBS and 6:2 FTS, and the long chain PFOA were more commonly detected and at higher concentrations than the MSE and USE despite similar total PFAS concentrations. Thus, it could be argued that the Swan Estuary has a greater influence on the PFAS concentration and composition in the LSCE than the Canning Estuary.

There were significant temporal patterns in the PFAS concentration and composition observed within the Swan Canning Estuary and catchment. During winter sampling (June 2017 and 2018), the concentration and spatial variation of PFAS within the estuary was greatly reduced relative to December 2016 and 2017. This pattern was strongly evident in June 2018 where concentrations had dropped substantially from the previous December and were remarkably consistent throughout the estuary. This reduction of PFAS in the estuary was despite more PFAS (relative to December 2016 or 2017) transported to the estuary via surface water as indicated by the increase in PFAS load at most gauged sites. The reduction in PFAS concentration and spatial uniformity in the estuary despite greater PFAS load was most likely a result of greater freshwater flow diluting PFAS concentrations within catchments discharging to the estuary (e.g. large reduction of PFAS at Airport North Main Drain in June 2018) and greater flow and dilution throughout the system. In summer (December 2016 and 2017) the reverse tended to occur, where PFAS concentrations were higher throughout the estuary despite lower PFAS load in the surface water drains. This suggests that groundwater recharge into the estuary, particularly throughout the MSE and USE, may be a substantial source of PFAS during the dry low flow period in summer and autumn (see also Ahrens et al. 2015). The exception to this was observed in the Ellen Brook where the connection of Ellen Brook US through to the confluence with the estuary during winter flow resulted in much higher concentrations at the Ellen Brook DS site and elevated concentrations in the USE. Furthermore, when Ellen Brook US was flowing the PFAS load was substantial and in June 2018, of the sites with operating gauging stations, it contributed the highest PFAS and PFOS+PFHxS load to the Swan Canning Estuary. While the evidence suggests surface flows are the major source or PFAS to the Ellen brook a recent groundwater modelling study (GHD 2018) on the PFAS contamination in the Ellen Brook catchment found that contaminated ground water from the Pearce Airbase may not yet be discharging into the Ellen Brook, suggesting possible legacy issues in this catchment could remain for many years.

A significant finding of this study was the higher concentrations of total PFAS throughout the estuary and catchment in December 2017, while the PFOS concentrations remained largely consistent. It was an increase in the shorter chained PFAS (PFBA, PFBS, 6:2 FTS, PFPA) that drove the increase in total PFAS. The increase in the environmental prevalence of these compounds may reflect the change use of PFAS compounds from the PFOS, PFHxS and PFOA based

compounds to industrial and commercial products containing shorter chained compound products. This may suggest some success in the widespread restriction of use of the longer chained compounds. However, the continued presence of PFOS and PFOA in the estuary and catchment sites despite a shift to using shorter chain substances may point to the legacy of historic widespread use of these compounds throughout the catchment (e.g. Ahrens et al. 2015). While a number of studies have identified that the longer chain PFAS (e.g. PFOS, PFHxS and PFOA) remain stable over time despite the reduction in use throughout the catchment areas (Ahrens et al 2015), others have found a distinct drop in long chain PFAS concentrations and a shift to the shorter chain PFAS (i.e. PFBS) (Hong et al. 2015). Such dynamics most likely result from both the nature of the historic use and groundwater hydrology. Interestingly, it was in the Canning Estuary basin (CE and LCR) where the short chain PFAS were most prevalent. This outcome was likely a result of current industrial uses of these compounds in the light industrial areas within the Canning catchment. However, in the Swan Estuary, PFOS and PFHxS were the dominant compounds detected in this study reflecting the legacy AFFF contamination issues from the sites draining Perth Airport (Airport North and South Main Drains) and Pearce Airbase (Ellen Brook).

Due to the historic long-term use of the PFAS within the catchment of the Swan Canning Estuary and the contamination of soils and groundwater (Ascot 2018, GHD 2018), it is likely that low level exposure to PFOS and PFHxS in particular, will be on going. A recent study by Ahrens et al. (2015) into stream and lake PFAS contamination from a nearby airport showed consistent concentrations of PFOS over time despite no reported use of PFOS for the previous 10 years. They suggested that the major source was contaminated groundwater slowly discharging into the river and lake (Ahrens et al. 2015). It is important to note that the presence of short chain PFAS, particularly in the Canning Estuary, highlights the ongoing use and environmental release of fluorinated compounds in industrial and commercial processes throughout the catchment. Additionally, the elevated concentration of predominately short chained compounds observed in Bickley Brook in December 2017 and not any other sampling periods may be indicative of a short-term release, perhaps associated with the rainfall event in the time preceding sampling. Elevated concentrations of PFAS associated with single spill events do not tend to remain within the water column at a for an extended period. Follow up sampling of spill events have observed rapid loss of PFAS within 120 days (Oakes et al. 2010), indeed the following sampling period in June 2018 did not detect elevated concentrations of these compounds. Even elevated concentrations in biota have been shown to decline rapidly post contamination event (Taylor and Johnson 2016). Thus, the ongoing persistent of legacy PFAS contamination is likely to be the ongoing cause of PFAS contamination with the estuary.

6.2 Biota

Per- and poly-fluoroalkyl substances (PFAS) were detected in all black bream (*A. butcheri*) and blue swimmer crab (*P. armatus*) specimens collected from the Swan Canning Estuary. Consistent with a broad range of studies, PFOS and PFHxS were

the most prevalent PFAS within all tissue types (e.g. Gaylard 2017, Vijayasarathy et al. 2017, Taylor et al. 2018). However, the contribution of PFOS to the total summed PFAS concentration was lower in the crabs than bream, with PFOA, PFHxS and the longer chained PFAS including PFNA, PFDA and PFDoA more prevalent than in the fish. The short chain PFAS compounds such as PFBS, PFBuA, PFPeA and 6:2FTS were not detected in any crab or bream samples. In all comparative tissue types, the PFAS concentrations in *P. armatus* were generally much higher than in *A. butcheri*. This is a pattern also noted by others (Taylor and Johnson 2016, Taylor et al. 2018), who suggested that differences in gill physiology, metabolism, and dietary pathways may contribute to higher PFAS concentrations in crustaceans than fish. For example, benthic feeding by the crabs may result in the ingestion of contaminated sediments and increased exposure to bound PFAS. Additionally, in the current study, the concentrations in *P. armatus* viscera (hepatopancreas, gills and gonads) were 5-10 times higher than that in the muscle, suggesting these organs are sites of either PFAS storage, exposure or depuration.

The partitioning of PFAS compounds in the different *A. butcheri* tissues was largely consistent with the literature. It has been reported that blood, liver and gonads (depending on maturation stage) are generally highest in PFAS concentration, followed by liver and muscle (Martin et al. 2003, Sharpe et al. 2010, Falk et al. 2015, Taylor and Johnson 2016). The partitioning patterns are reflective of the preference of PFAS to bind to proteins rather than lipids, as many other organic contaminants do (Walters et al. 2016). Female bream had far higher gonadal PFOS+PFHxS concentrations than male fish, which likely reflected preferential binding of PFAS to proteins in ova rather than testes and the advanced maturation stage of the fish (Sarre and Potter 1999). The time of sampling was well within the peak reproductive period of black bream (Sarre and Potter 1999), and in the lead up to reproductive activity fish often consume greater abundance of food resources and this is often sequestered in gonads. The greater dietary exposure to PFAS combined with ambient water exposure and the partitioning of resources to gonad development is the likely cause of the greater PFAS concentration and burden in female fish at this time.

The crabs exhibited a similar pattern of PFAS partitioning, with higher PFAS concentrations in the viscera, which included the hepatopancreas, gonads and gills. The PFAS concentrations were also far higher in the female crabs. Additionally, the sampling period encompassed the key reproductive period for the crabs (de Lestang et al. 2003). Consequently, the higher concentrations in female crabs especially in the viscera and the timing of the sampling encompassing the key reproductive period suggests PFAS uptake and storage in the gonads was occurring in female crabs, at a greater rate than male crabs. Female crabs spawn in September to December and can produce multiple batches over the spawning season, thus gonadal development would be occurring in the crabs sampled (de Lestang et al. 2003). While the female crabs spawn from September to December, the crabs do not mate until January-February and the females storing the spermatozoa until the following spring-summer spawning period (Potter and De Lestang 2000, de Lestang et al. 2003, Harris et al. 2016). Thus, male crabs would be undertaking gonadal development at this stage.

The much greater concentrations of PFAS in the ovaries in comparison to testes during the reproductive period suggests that PFAS binds far more readily to proteins in the ova then those in spermatozoa.

While the PFAS concentration was lower in the male crabs, the male PFOS+PFHxS body burden increased significantly with increasing carapace width, a trend not observed in the female crabs. In the bream, PFAS burden increased with increasing length in female fish only. It is suspected that reproductive effects occurring at the time of sampling may be responsible for this result. For example, it is not likely that the male crabs had mated at the time of sampling, resulting in an accumulation of PFAS bound to proteins in the testes, while female crabs may have undergone multiple spawning events. Maternal transfer of PFAS from the body of the crab to the eggs maybe occurring (Peng et al. 2010), which can result in the loss of PFAS from the body of the female (Sharpe et al. 2010). Larger crabs produce a greater number of eggs (de Lestang et al. 2003) and thus PFAS loss may be proportional to size (and egg batch size). It could be argued that the female crabs did not show an increase of PFAS burden with size due to spawning and material transfer of PFAS, the capture of non-ovigerous crabs which were maturing ovaries may have resulted in the higher concentration and burden in females than males. The male crabs had not yet mated and thus were had accumulated PFAS. In the bream it could hypothesized that once spawning had occurred the PFAS burden in the females would decrease.

This study has demonstrated an increase in PFAS body burden with size in male crabs and female bream. However, there was no significant increase in PFAS concentration with body size in either the bream or crabs. While it is acknowledged that only adult fish and crabs were sampled in this study, it was expected that the concentration of the known bio-accumulative substances, PFOS+PFHxS, would increase with the size of the individual. The consistency of the PFOS+PFHxS concentrations in the fish and crab tissue, regardless of size, suggests some capacity for these species to regulate the concentration of these compounds within their body. This is a finding that is emerging in research on environmental consequences of PFAS contamination, for example, Gewurtz et al. (2014) found that fish size had no impact on the concentration of PFOS across multiple species, while (Baduel et al. 2014) found that hepatic PFOS concentration in stingrays declined with increasing size. While reproductive activities may be a key contributor to the accumulation and loss of PFAS from the body of aquatic species there may be further mechanisms contributing to this result.

The ability of aquatic species to regulate and remove PFAS from body tissues has been reported to varying extents in a number of studies. Recently Taylor et al. (2018), in a study of crustacean exposure and depuration of PFAS, found that prawns were able to remove PFAS from body tissues rapidly, resulting in complete depuration of PFHxS within 50 hours and a depuration half-life of 158.5 hours for PFOS. Conversely, the crabs examined in the study showed much greater variation in depuration rates, particularly of the long chain PFAS. Investigations in fish have also found varied rates, for instance, very slow depuration in juvenile rainbow trout (Martin et al. 2003), while interestingly, Falk et al. (2015) found rapid depuration in

adult trout. Additionally, Falk et al. (2015) investigated the uptake and depuration of the short chain compound PFBS, which was widely detected in the estuary in the current study. They found PFBS was rapidly taken up by trout and reached a high concentration in body tissues particularly the liver, but when exposure stopped, the depuration was the most rapid (72% reduction in 3 days) out of the measured PFAS compounds (Falk et al. 2015). In the current study, if the species tested have a capacity to depurate PFAS rapidly (arguably more likely in the fish than crabs) then the concentration in body tissues may be indicative of the environment they had been recently inhabiting (capture site).

The rapid depuration rates of PFAS from some fish and crustacean species has been related to the constant interaction between the water and gills (Martin et al. 2003) and the high solubility of PFAS in water. Taylor et al. (2018) suggested that once concentrations within the gills exceed a theoretical solubility threshold, PFAS would diffuse back into the water across the gill membrane. This also provides a possible explanation for generally much higher concentrations and greater accumulation response of PFAS in air breathing mammals (e.g. Gaylard 2017). Gaylard (2017) found hepatic PFAS concentrations in dolphins from the Swan Canning Estuary more than 150 times higher than the bream or the viscera PFAS concentration in the crabs. PFAS does not diffuse from the lungs into the air as readily as to water and thus the lungs are not an effective mechanism for the regulation and depuration of PFAS in the body (Kelly et al. 2009). While diet has also been identified as a critical source of PFAS exposure, the binding of PFAS to blood serum may still allow the gill mediated loss of PFAS accumulated through dietary exposure.

Different regions in the estuary were targeted for biota collection. Regional differences in PFAS concentration were, however only observed in female *A. butcheri*, which had significantly higher PFOS+PFHxS concentration in the MSE than the CE. One possible explanation was the much greater extent of elevated surface water concentrations in the MSE and USE (SUC to STJ = 11 km) than the CE (CASMID to RIV = 2.4 km). Thus, we hypothesise that there was greater exposure risk to fish in the MSE and USE than the CE. In addition, PFOS and PFHxS were both more prevalent in the MSE than the CE. Females did have higher concentrations than the males and perhaps in the Swan Estuary, with greater exposure risk, assimilated a greater concentration than fish elsewhere. In addition, we found that the female gonads (ova) had significantly higher PFAS concentrations than the testes and contributed far more to the whole fish body burden.

In considering the impact of the different regions on fish and crab exposure to PFAS it is necessary to understand the possible residence time or site fidelity of biota in the regions of interest. In a short-term acoustic study on the movement of *A. butcheri* in the Swan Canning Estuary fish exhibited a reasonable degree of residence within the Ecological Management Zones, particularly within the MSE and USE (Watsham 2016). Fish were reasonably mobile within the EMZs with an average daily movement of 0.54 km. There were occasions of rapid movement between the EMZs which certainly suggest the ability to move throughout the system. Of additional interest to the current study was the detection of three fish (from 55 tagged fish)

which had moved between the estuary basins, from the MSE and USE to the CE (Watsham 2016). The degree of movement recorded suggests that fish may move between EMZs and occasionally, the estuary basins and that fish captured in the Canning or Swan Estuary may have been exposed to PFAS in a different estuary region. However, due to the spatial extent of the elevated concentrations in the MSE and part of the USE, and the apparent residence of fish to these regions (95% of tagged fish remained in the respective estuary basin), combined with the rapid uptake and depuration of PFAS by fish (Falk et al. 2015) the concentrations detected in this study may well represent exposure within a single EMZ.

The blue swimmer crab, *P. armatus,* is also known to exhibit a fair degree of movement within the Swan Canning Estuary. A mark-recapture study by Harris et al. (2016) in the Swan Canning Estuary determined that both male and female crabs tended to move upstream during the summer months when salinity and water temperature are increasing in the middle reaches of the system. Crabs were commonly found in the CE and MSE in mid to late summer, before retreating to the LSCE as the salinity of the middle estuaries declines due to the onset of winter flows (Harris et al. 2016). In the current study, *P. armatus* were sampled in December 2017 in the early stages of a likely upstream migration (Harris et al. 2016). As a result, despite significant effort, insufficient numbers were captured in the MSE and CE for any robust regional comparison. Consequently, the concentrations of PFAS in crab tissue could be expected to be conservative and perhaps not representative of exposure in the regions of the estuary with more elevated PFAS concentrations. Despite the typically lower concentrations of PFAS in the LSCE, the PFOS+PFHxS concentration in the body tissue of the crabs was consistently higher than the bream and exhibited a greater number of detects of longer chained PFAS. The higher concentrations in the crabs as opposed to the bream, may reflect the benthic feeding behaviour of the crabs and the potential to ingest sediment that may have bound higher concentrations of PFAS due to the more saline water. Blue swimmer crabs may also, metabolically, have a capacity for greater uptake and storage of PFAS material than the bream. Conversely, the bream may be far more effective in depurating body tissues of PFAS compounds. There is some evidence that suggests concentrations are generally higher in the crustaceans than teleosts (Vijayasarathy et al. 2017, Taylor et al. 2018), but it is certainly not a ubiquitous observation (Hong et al. 2015, Gaylard 2017). In a recent Australian example, an analysis of PFAS in mud crabs and fish in Darwin Harbour, Northern Territory revealed highly variable concentrations of PFOS+PFHxS in crab muscle and viscera, but mean concentrations were markedly similar to that detected in the blue swimmer crabs (a member of the same subfamily, Portuniae) in the current study (Vijayasarathy et al. 2017). Interestingly, PFHxS and PFOS concentrations in two of the three fish species sampled in the Vijayasarathy et al. (2017) study, the catfish and golden snapper, were considerably variable but generally less than the mud crabs for comparable tissue types. The exception was the top order predator, barramundi (*Lates calcarifer*) of which the concentrations were much higher than the other fish and in the same range as the mud crabs. The surface water concentrations at the biota collection sites were largely consistent with those recorded in the LSCE (Coffey Services Australia 2018).

The direct acute impacts (e.g. mortality or significant impairment) of PFAS exposure to biota is varied and, in some cases, dependant on very high concentrations. For example, 0.31-17.95 mg/L PFOS caused mortality in macroinvertebrates after short term exposure (Ji et al. 2008) and PFOS concentrations between 1-5 mg/L caused significant reductions in hatch rate and growth in zebrafish (Shi et al. 2008). However, there is a growing body of evidence to suggest chronic effects at much lower concentrations (i.e. 2-20 µg/L) may be expressed in subsequent generations and result in reproductive impacts and reduced fitness in fish (Keiter et al. 2012, Lee et al. 2017) and invertebrates (Stefani et al. 2014, Jeong et al. 2016). These impacts may also be cumulative in subsequent generations (Ji et al. 2008). While the concentrations of PFAS that were used in these studies were still elevated in comparison to those concentrations typically found in the Swan Canning Estuary, they still provide some insight into the potential impact of these substances in the environment. Furthermore, it is challenging to determine the actual extent of the current impact on biota and ecosystem function within the Swan Canning Estuary given the system has been exposed to historical PFAS over a number of decades (Department of Defence 2007). As a result, any biota in the estuary and catchment has been exposed to PFAS for many generations and any particular effects from these substances was likely already occurring within the population.

Recently, the synergistic, additive and in some cases antagonistic effects of PFAS with other PFAS compounds and with other common aquatic contaminants have been demonstrated (Kim et al. 2011, Rodea-Palomares et al. 2012, Ahrens and Bundschuh 2014, Du et al. 2017). Given the known contaminants in the Swan Canning Estuary include heavy metals, PCBs, PAHs, and organochlorine pesticides (Nice 2009, Nice et al. 2009, Hoeksema 2015), it is possible that significant synergistic or additive effects may be experienced by estuarine organisms in the system. In addition, the zones of the estuary with the highest surface water PFAS concentrations including the Middle Swan Estuary and upper Canning Estuary, also had the highest concentrations of other contaminants, albeit in the sediment (Nice 2009). Thereby multiple stressor approaches are increasingly recognised as the best approach to understanding contaminant impacts on aquatic ecosystems (Letcher et al. 2010, Craig et al. 2017).

An investigation conducted by the South Australia EPA examining PFAS concentrations in the livers of dolphins from various populations throughout Australia indicated that those from the Swan Canning Estuary had the highest concentrations observed both in the study and globally (Gaylard 2017). This work is currently being reviewed and expanded by Murdoch University with outcomes due in 2020. Data collected in the current study indicated that water concentrations in the Swan Canning Estuary were typically higher than those recorded in the Port River Estuary and Barker Inlet in South Australia (Gaylard 2017), particularly those from the December 2017, where concentrations of PFOS and PFHxS where more than ten times higher throughout the Middle Swan Estuary and Canning Estuary. The concentrations of PFAS in the black bream and blue swimmer crabs in the current study were also higher than those for fish and crabs collected in the South Australia investigation (Gaylard 2017) and to those in comparative studies in New South

Wales (Taylor and Johnson 2016, Taylor et al. 2018), but were similar to those in a study of fish and crabs in Darwin Harbour (Vijayasarathy et al. 2017). The difference in concentrations between the fish in the current study and Gaylard (2017) was not as dramatic as that between the concentrations in dolphin livers. As the current study did not seek to determine the trophic transfer of PFAS within the Swan Canning Estuary, it is difficult to comment on potential impact of the observed concentrations of PFAS in water and biota on higher order consumers, such as dolphins.

6.3 Consumption and health guidance

Based on the methodology established in Hoeksema (2015), the concentrations of PFOS+PFHxS, and PFOA in the edible portion of both the black bream and blue swimmer crabs were not sufficient to restrict consumption over an average person's lifetime. These results are supported by a recently completed and detailed Human Health Risk Assessment for potential PFAS exposure from the Swan Canning Estuary (Department of Health 2020) which applied a different method to determine the acceptable exposure limits. The consistency in result between the two studies, whilst applying different methods strengthens the conclusion that there is negligible risk to the human population from consumption of black bream and blue swimmer crabs from the Swan Canning Estuary.

The data collected in this study suggest that there is negligible human health risk from PFAS exposure from swimming in the Swan and Canning Estuary. Recreational water quality guidelines for PFOS+PFHxS and PFOA were not exceeded at any site throughout the Swan Canning Estuary and therefore primary contact with the surface waters in the estuary does not present a risk to the community. The recreational water quality guideline for PFOS+PFHxS were however, exceeded at Airport North Main Drain on three of the four sampling occasions (excluding June 2018). Perth Airport Pty Ltd, in summer 2019-20, commenced treatment of summer flows from Perth Airport North Drain using a granular activated carbon treatment system. The treatment system is capable of treating the entirety of summer flow with the ability to substantially reduce PFAS concentrations within the drain and potentially the estuary. Given the low likelihood of single or repeated recreational activities in the proximity of this sampling site (as a listed contaminated site the location is fenced and access restricted), additional precautionary advice has not been issued by DoH.

7 Conclusions

Given the current understanding of PFAS contamination globally, it was not unexpected that PFAS were detected throughout the estuary, catchment and in the body tissues of key aquatic species. While the concentrations in the surface water and biota appeared higher than some comparative examples nationally, the draft PFOS AWQG for 95% species protection was exceeded only within the catchment sites draining known sources of PFAS contamination. Thus, given current available data the risks to the estuary from PFAS contamination would be considered low. However, there remains a lack of indicative aquatic biota health guidelines in

Australia and as such the effects of PFAS contamination on biota were difficult to ascertain. While PFOS and PFHxS were the most prevalent singular compounds throughout the estuary, the widespread detection of short chain PFAS is likely reflective of contemporary uses of such compounds. Short chain PFAS compounds such as PFBS, PFBA, PFPeA and 6:2FTS are generally considered an environmentally safe alternative to the longer chain PFAS (Department of Health 2018). While these compounds are acknowledged as being persistent in the environment, they are not known to bioaccumulate and both acute and chronic effects are considered unlikely due to improbably high concentrations required to cause effects (Department of Health 2018). However research into the short chain compounds PFBS is beginning to identify toxic effects to both fish (Chen et al. 2018) and invertebrates (Stefani et al. 2014). Thus the rapid increase in their prevalence within the estuary suggests some caution ought be applied to their widespread application (Ahrens and Bundschuh 2014).

Body tissue contributions to total PFAS body burden largely conformed to known tissue partitioning patterns. With the liver and viscera (hepatopancreas, gills and gonads) having the highest concentration in the bream and crabs, respectively. The PFAS concentrations in the female gonads were substantial and contributed a significant proportion to the total PFAS body burden. Conversely, the male gonads had much lower concentrations and did not make a great contribution to total PFAS body burden. Females had higher PFOS+PFHxS concentrations in the Middle Swan Estuary then females in the Canning Estuary. This result suggested that fish may have had greater exposure to PFAS in the Middle Swan Estuary and, due to the advanced maturation stage of gonads, may have resulted in greater uptake of PFAS from the water.

The concentrations of PFAS in the surface water of the estuary at the monitored sites do not present any risk to the human population from recreational exposure. The consumption of fish and crabs, likewise present minimal risk to human health from consumption.

Appendices

Appendix 1 Procedures to avoid cross contamination during the collection of samples for PFAS analysis

In accordance with the Department of Environment Regulation (DER 2016) following practices were adhered to during all PFAS sampling and laboratory processing:

- Sun cream was not worn – physical barriers were used instead i.e long sleeved shirt, face and neck sleeve, wide brimmed hat, long pants and gloves (nitrile gloves).
- All clothing must be more than six washes old
- Hand creams, moisturisers and make up were not worn
- No plastic or foil packaged food or drink with a non-stick internal barrier was permitted.

Water

Cross contamination was a significant risk in the sampling for PFAS in surface waters. To avoid cross contamination the protocols developed by the Department of Environmental Regulation (Department of Environment Regulation 2016) were strictly adhered to. Before sampling commenced, samplers were assigned roles, one person was the sample collector, the other was assigned the “clean-hands” role. At the commencement of the sampling day both samplers washed their hands and forearms with soap, then PFAS free deionised water, dried their hands with a clean paper towel and put on clean nitrile gloves. At each site and before sample collection, the clean hands sampler put on new nitrile gloves and then provided the sample collector with clean gloves and a sample bottle (HDPE with no PFTE liner provided by the laboratory). When samples were collected on foot, the sampler entered the water downstream of the intended sampling location and walked slowly into the flow. Once at the desired location, the sampler submerged the sample bottle into the water, cap first. When fully submerged the bottle was then righted and faced into the direction flow, the cap removed, and the bottle filled. Before the bottle was removed from the water, the cap was screwed on tightly. The sampler then retuned the sample to the clean hands sampler, who dried the bottle with clean paper towel. The sample collector then removed their gloves, washed their hands with deionised water, dried them with a clean paper towel and given a new pair of gloves. The completed label was then adhered to the bottle, which was then double bagged (in food grade snap-lock HDPE bag) and stored in a clean esky on double bagged ice. The gloves were then worn whilst travelling to the next site. For samples that were collected on the boat, the boat was kept gliding in gentle motion with the engine off while the sample was collected. This provided gentle flow that the bottle was pointed in to and reduced the risk of any potential cross-contamination from the sampler or vessel.

Biota

Sample collection

Bait was sourced frozen in bags from a commercial supplier and so handling of the fish in accordance with PFAS hygiene protocols couldn't be assured.

All handling of crabs was done using clean nitrile gloves which were replaced before emptying every trap. The traps were hauled by hand onto the boat. Once the trap was at the water surface it was gently shaken in the water to remove any debris and emptied directly into an ice bath. The ice bath consisted of an esky that had been thoroughly scrubbed with a HDPE scrubbing brush and ethanol. It was rinsed with deionised water, then site water before being filled with site water. In each EMZ the ice bath was rinsed and the water replaced. Ice was double bagged with HDPE food grade snap lock bags and placed into the esky to cool the water. Once the crabs were anaesthetised they were measured to ensure they were above the minimum legal limit (127 mm CW), sexed, double bagged (in food grade HDPE snaplock bags) and euthanised by placing on ice (double bagged) in a clean storage esky (washed with ethanol, rinsed with deionised water). The ice bath consisted of an esky that had been thoroughly scrubbed with a HDPE scrubbing brush and ethanol. It was rinsed with deionised water, then site water before being filled with site water. In each EMZ the ice bath was rinsed and the water replaced. Ice was double bagged with HDPE food grade snap lock bags and placed into the esky to cool the water.

All fish were handled using clean nitrile gloves changed before handling from different each seine. The ice bath consisted of an esky that had been thoroughly scrubbed with a HDPE scrubbing brush and ethanol. It was rinsed with deionised water, then site water before being filled with site water. In each EMZ the ice bath was rinsed and the water replaced. Ice was double bagged with HDPE food grade snap lock bags. Once fish were euthanised, they were doubled bagged, placed on ice in a clean storage esky and transported to the laboratory for processing.

Laboratory processing

Before biota samples were processed, the laboratory bench space was scrubbed with ethanol, rinsed with deionised water and dried with clean paper towels before a clean HDPE plastic sheet was used to cover the entire work surface. Nearby work benches that could provide a potential contamination risk but couldn't be removed from the area were covered with clean HDPE sheets. The immediate work area including balance, processing board and sink were covered with an additional HDPE sheet which was changed with every specimen. All dissecting implements that were in contact with sample material, such as scissors, knives and forceps, were washed with ethanol and rinsed with deionised water after every specimen. Nitrile gloves were used throughout sample processing and new gloves were worn for each sample.

It is widely reported in the literature that the muscle tissue generally has the lower concentration of PFAS (e.g. Martin et al. 2003, Gaylard 2017). As a result, crab and bream samples were processed in a manner to avoid the potential cross

contamination from other organs. For this reason, the muscle samples were processed first. Additionally, the crab muscle sample comprised of the muscle from the chelipeds and pereopods only. There was an unacceptable risk of contamination of the body muscle tissue from the viscera and gastric juices.

The bream were processed in similar way, with the muscle (fillets) removed first with great care taken to not penetrate the body cavity. During removal of the gonads and liver, care was taken to avoid rupturing the any internal organs

Each bream tissue sample (i.e. fillet, carcass, liver, gonads) was rinsed with deionized water, dabbed dry with clean paper towel, before storage in a lab supplied clean HDPE zip lock bag. The sample was double bagged and then frozen. The crab samples were extracted and placed directly into the sample bag which was then double bagged and frozen.

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