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Do conventional cooking methods alter concentrations of per- and polyfluoroalkyl substances (PFASs) in seafood?

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ABSTRACT

Per-and poly-fluoroalkyl substances (PFASs) are bioaccumulative chemicals of emerging concern. Some PFASs accumulate in seafood, and can contribute to dietary exposure. Previous work has suggested cooking seafood decreases concentrations of neutral organic contaminants, however, previous studies dealing with PFASs have shown conflicting results. In this study, the potential changes of PFAS concentrations as a result of boiling, frying and baking are systematically examined. Blue Swimmer Crab (*Portunus armatus*), Dusky Flathead (*Platycephalus fuscus*) and School Prawn (*Metapenaeus macleayi*) were obtained from near a known PFAS point source and a reference location (affected by diffuse sources). Raw and cooked samples were analysed for commonly found PFASs. Of 23 target analytes, PFOS was the most frequently detected compound. PFOS, PFHxS and PFOA concentrations in School Prawn effectively doubled after boiling, and PFOS increased when Dusky Flathead was baked. There was no significant difference in PFOS concentration when Dusky Flathead was fried, or when the Blue Swimmer Crab was boiled. PFHxS and PFOA concentrations in Blue Swimmer Crab effectively halved after boiling. Increase in PFAS concentrations possibly arise from mass loss during the cooking process. Our data show that cooking does not consistently reduce PFAS concentrations, and cannot mitigate dietary exposure.

1. Introduction

Per- and poly-fluoroalkyl substances (PFASs) are a class of chemicals with unique properties, that have a broad suite of applications in industrial processes and consumer products (Kotthoff et al., 2015). These compounds are contaminants of emerging concern, particularly due to their persistence in the environment, bioaccumulative properties, and potential toxicity in animals. High PFAS concentrations are being increasingly detected around industrial sites, airports, and military bases (e.g. Baduel et al., 2017; Hu et al., 2016), as well as other diffuse sources (e.g. Alder and van der Voet, 2015; Perkola and Sainio, 2013). These compounds are readily transported in groundwater and surface water, which often results in adjacent aquatic ecosystems being impacted (e.g. Nakata et al., 2006; Taylor, 2018; Yoo et al., 2009).

The literature on the effects of PFASs on humans is inconclusive, however testing on animals has shown some effects at low doses (FSANZ, 2018). Reported potential health effects in humans include increased levels of cholesterol and uric acid in the blood and reduced kidney function, although the level of health effect reported in people with the highest exposure is generally still within the normal ranges for the whole population (Expert Health Panel for Per- and Poly-fluoroalkyl Substances, 2018). Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA, both perfluoroalkyl acids or PFAAs) in particular have shown to be widely distributed in the environment and bioaccumulate in both humans and wildlife (Ahrens, 2011; Martin et al., 2013). PFOS has been listed for restriction under the Stockholm Convention on Persistent Organic Pollutants (Wang et al., 2009), and PFOA and PFHxS are under consideration for listing. In recent years, various Health Based Guidance Values (HBGVs) have emerged for some PFAAs (e.g. FSANZ, 2017; Knutsen et al., 2018; US EPA, 2017), and while some of these HBGVs differ, many jurisdictions are developing strategies to manage exposure risk for this group of chemicals.

Exposure risk for PFASs is greatest through ingestion of contaminated drinking water (e.g. Hölzer et al., 2008) or consumption of contaminated plants or animals (e.g. Chen et al., 2018; Fromme et al., 2009; Vestergren and Cousins, 2009). Depending on risk assessment outcomes, this can lead to advice that suggests limiting consumption of produce or animals that have been exposed to the contaminated media,

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or avoiding consumption all together. Where PFASs impact aquatic species, consumption of aquatic animals can represent a significant PFAS exposure pathway (Noorlander et al., 2011). Consequently, risk management measures can also require advice on consumption of aquatic biota in ecosystems adjacent to contaminated locations.

A diverse range of freshwater and marine aquatic biota have been shown to bioaccumulate PFASs, especially perfluoroalkyl acids (PFAAs) with 6 or more fluorinated carbons (e.g. Baduel et al., 2014; Ding and Peijnenburg, 2013; Fang et al., 2016; Martin et al., 2003; Naile et al., 2010; O'Connor et al., 2018; Taylor, 2019; Taylor and Johnson, 2016). While bioaccumulation of PFAAs may create ecological risks for aquatic species and species that consume them (such as birds, reptiles and mammals), many species are also exploited through commercial and recreational fisheries. These exploited species are usually consumed directly (i.e. by recreational and commercial fishers) or on-sold into the food supply (by commercial fishers). In estuarine systems, seafood generally includes mollusc, prawn (=shrimp), crab and fish species (Taylor, 2019; Taylor et al., 2018). The exposure risk for the general population is usually relatively low since food generally comes from a wide variety of sources, but residual exposure risk may remain for fishers who consume large amounts of locally sourced product. Thus, the investigation of other factors that could mitigate this residual exposure risk is particularly relevant for certain groups in the population.

While some estuarine species are consumed raw (such as oysters), most are cooked prior to consumption. Cooking has been shown to influence the concentration of heavy metal and other organic contaminants in fish and seafood. For example, cooking resulted in the reduction of dioxin compounds in Carp (Stachiw et al., 1988) and Mackerel (Hori et al., 2005), and similarly resulted in a reduction of polychlorinated biphenyls, polybrominated diphenyl ethers and other persistent organic pollutants (POPs) in Salmon (Bayen et al., 2005). The latter study demonstrated that loss of POPs was directly correlated with loss of lipid from tissues during the cooking process. However, this is not a universal effect, and the outcome depends on the chemistry of the contaminant, the cooking process employed, and the species examined (Domingo, 2010). Del Gobbo et al. (2008) presented data indicating that cooking could decrease concentrations of PFAAs in the edible tissues of marine and freshwater fish, although the study may have been confounded by comparatively low concentrations of PFAAs. Later work by Bhavsar et al. (2014) and Vassiliadou et al. (2015), suggested that cooking muscle tissue of marine and freshwater fish species was not an effective approach to reduce dietary exposure to PFAAs. Most recently, Sungur et al. (2019) reported substantial reductions (50–90%) in fish muscle PFAA concentrations following cooking. These conflicting reports highlight the need for further investigation on the influence of cooking on concentrations of PFAAs, and whether the process of cooking can mediate exposure to PFASs in at-risk groups.

The chemical properties and stability of PFAAs (e.g. the stability of the carbon-fluorine bond) means that degradation of these compounds due to the cooking process is unlikely. However, it is possible that concentrations could be impacted by losses to cooking media or moisture losses during cooking (Krafft and Riess, 2015). Also, if precursors to PFAAs are present in the fish tissues, these could possibly transform under cooking conditions to form PFAAs, and thereby influence the measurable concentrations. This study aimed to further examine the effects of common cooking processes on concentrations of PFASs in representative seafood species. The target PFAS analytes included a suite of PFAAs and a small suite of PFAA precursors.

2. Methods

2.1. Study design and sample collection

The core objective involved comparing PFAS concentrations in contaminated fish (collected from Hunter River, New South Wales) before-and-after cooking treatments, for each sample. Samples were collected from Fullerton Cove, which is the location of a significant point-source for PFAS contamination in the Hunter River Estuary (which originates from a nearby airport, Taylor and Johnson, 2016). Reference samples from Wallis Lake, New South Wales (subject to diffuse sources) were treated equivalently. Batch-tested cooking media was analysed for PFASs after cooking to evaluate the potential movement of PFASs out of contaminated animal tissue into the media during the cooking process.

Fish, crab and prawn samples were collected from the contaminated or reference estuary on the morning that each cooking treatment was to be carried out. Dusky Flathead and Blue Swimmer Crab were collected using a gill net, and School Prawn were sampled using an otter trawl. Following capture, samples were immediately placed within a labelled, snaplock bag and placed on ice. Samples were then transported to the laboratory for sample preparation and cooking within 1 h. Prior to cooking, morphometric data from each individual was measured.

2.2. Sample handing and preparation

Distilled water and cooking oil were both batch-tested prior to any work commencing, and verified that PFASs were below limits of detection. Crabs, prawns and fish were prepared and cooked following methods as used by a home cook, with additional procedures to ensure there was no additional contamination of samples with PFASs (e.g. use of stainless steel equipment and rinsing with batch tested water). Blue Swimmer Crab and School Prawn meat were cooked by boiling in salted ($8.5\ g\ L^{-1}$) water (500 mL), and Dusky Flathead samples were either baked or pan fried in olive oil. Biota tissue fractions (except crabs) and cooking media were weighed before and after cooking (note that for School Prawn this was the abdominal prawn meat for the composite). For the baked or fried treatments, the internal temperature of the cooked meat was measured immediately after it was removed from the pan or oven. Uncooked and cooked meat samples were thoroughly homogenised with a mortar and pestle. Cooking oil was collected in its entirety from the baked and fried samples, and a 50 mL subsample of cooking medium from each boiled treatment was placed in a Falcon tube for later analysis. For both contaminated and reference site samples, six replicates were cooked for each species and each relevant cooking treatment, with the exception of Dusky Flathead for Wallis Lake ($n = 5$).

For crabs, raw meat from the left part of the body of each crab was carefully removed, and used as the uncooked sample. The remaining crab portions were cooked separately for 7 min in a fresh batch of boiling salt water, and the maximum temperature of the cooking media was measured immediately prior to removing it from the hotplate. Crabs were left to cool and the cooked meat from the right part of the body was removed and prepared for analysis. Whole prawns were cooked in a similar fashion to crabs, but only boiled for 4 min, and each replicate consisted of a composite of 10 similarly-sized individuals. Thus, the uncooked and cooked samples comprised meat from 10 prawns that were peeled, deveined and de-headed.

For fish, the skin was removed and each side of the individual filleted (yielding two similarly sized fillets). Each of the two fillets was cut into three similar sized pieces, and one piece from each side was randomly allocated to the uncooked, fried and baked treatments. Two pieces (i.e. one left and one right fillet) of each fish were pan fried in 40 mL of hot batch tested olive oil (Cobram Estate, Victoria, Australia) for 2–3 min (dependent on the thickness of the fillet and the internal temperature). Similarly, two pieces (i.e. one from the left and right fillet) of each fish were baked for 10 min in 20 mL of olive oil. Samples from each individual were cooked separately, and all equipment thoroughly cleaned with batch-tested distilled water between each sample.

2.3. Sample extraction and analysis

2.3.1. Chemicals and reagents

Target analytes included perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTriDA), perfluorotetradecanoic acid (PFTreDA). perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), perfluorooctanesulfonic acid (PFOS), perfluorodecanesulfonic acid (PFDS), fluorooctanesulfonamidoacetic acid (FOSA), N-ethyl perfluorooctanesulfonamidoacetic acid (NEtFOSAA), N-methyl perfluorooctanesulfonamidoacetic acid (NMeFOSAA), N-methyl perfluorooctane sulfonamidoethanol (NMeFOSE), N-ethyl perfluorooctane sulfonamidoethanol (NEtFOSE), and the fluorotelomer sulfonates 8:2 FTS, 6:2 FTS, and 4:2 FTS.

Mass-labelled extraction standard was used to be able to account for method recovery. This standard mix included ^{13}C and ^{18}O labelled PFAS (purchased from Wellington Laboratories, Guelph, Ontario, Canada). For PFASs that did not have corresponding mass labelled standards, standards with the closest and most similar structure were used to quantify the native PFASs. Instrumental recovery standard was used for quantification and included $^{13}C_8$-PFOA and $^{13}C_8$-PFOS. Further details on the chemicals and reagents are presented in the supplementary information.

2.3.2. Extraction

Prior to extraction, all samples were spiked with 0.2 ppm mass-labelled PFAS standard mix. Homogenised biota tissues were extracted according to (Baduel et al., 2014). Briefly, 1 g of tissues were digested by 0.4 mL 200 mM sodium hydroxide in methanol (MeOH) and extracted with 4 mL acetonitrile. Clean-up was performed by liquid-liquid extraction using n-hexane, followed by samples being pushed through carbon cartridges (Bond Elut, 100 mg, Agilent Technologies). Water samples (50 mL) were extracted using a solid phase extraction method specified by (Thompson et al., 2011). Strata X-AW cartridges (Phenomenex) were used for extraction and samples were eluted with 4 mL 0.2% ammonia:methanol solution. Further clean-up was performed by percolation through carbon cartridges (Bond Elut, 100 mg, Agilent Technologies). Oil samples (0.3 mL) were extracted using 0.6 mL of MeOH/H_2O 0.5% NH_3H_20 and DCM, according to the method established by Tang et al. (2014). Prior to instrumental analysis, all samples were spiked with 10 µL 0.2 ppm instrument standard. Further details on extraction methods and PFAS standard mixes are presented in supplementary information.

2.3.3. Instrumental analysis

PFAS analysis was performed using a high performance liquid chromatography (HPLC, Nexera HPLC, Shimadzu Corp, Kyoto Japan) coupled to a tandem mass spectrometer (SCIEX QTrap 5500 and 6500 +, Concord, Ontario, Canada) with negative electrospray ionisation mode and multiple reaction monitoring (MRM) mode. The injection volume was 5 µL. To separate the target PFASs, a gradient elution of mobile phase 1% (Aqueous Phase A) and 90% (Organic Phase B) MeOH, respectively, with 5 mM ammonium acetate were used. The column was a NX C18 column (50 X 2 mm, 3 µm, 110 Å, Phenomenex, Lane Cove, Australia), and the temperature was held constant at 50 °C. Integrations of peak areas were performed using MultiQuant (3.0.2). Retention times and comparison of MRM transitions between samples and the calibration was used for confirmation and identification of peaks. Calibration standards (linear isomers of each compound, 500 µL; 200 µL methanol and 300 µL 5 mM ammonium acetate in water) had a concentration range of 0.1–100 µg L^{-1} (0.1; 0.4; 1; 4; 10; 20; 40; 60; 80; 100). When the sample concentrations exceeded the calibration range, dilutions of the samples were made, and run again.

2.3.4. Quality assurance

Samples were extracted in batches of 12 samples. In each batch, a blank (ACN/olive oil/MilliQ) was included to be able to detect potential contamination. No PFAS analytes were detected above limit of quantifications in any blanks. For each batch a non-extracted side spike was analysed together with the samples. In batches of biota and oil samples, additional quality controls included a duplicate sample and native spiked duplicate, which both followed the same extraction procedure as the samples. In batches of water samples, a native spiked blank was used instead, as the amount of sample was not enough to make up duplicates. The recovery of the native spike was determined by subtracting the analyte concentrations detected in the spiked samples by the analyte concentration found in the unspiked duplicate sample. In each batch of biota samples, a QAQC matrix sample with a known PFAS concentration followed the same extraction procedure as the samples and used to calculate between batch-variation. Details of native recovery and QAQC matrix variations are presented in supplementary information.

During instrumental analysis, calibration standards were injected twice in each run. Quality control standards were added between every 10 samples to monitor instrumental variations. Linear (weighted by 1/x) or hill regression fits were applied on the calibration curves for each linear isomer, and were used to quantify the analytes. Regression coefficients (R^2) were only accepted > 0.993. Limits of detections (LODs) and were set three times the standard deviation of the concentration of the lowest standard after 10 injections of the standard. Limits of quantification (LOQs) were set 10 times the standard deviation and ranged from 0.05 to 3 µg kg^{-1} for different PFASs in biota and oil, and 1–12 ng L^{-1} for different PFASs in water. Further details are presented in supplementary information.

2.4. Statistical analyses

Concentrations of PFAS in cooking media samples were qualitatively examined to identify if PFASs were liberated from the biota tissue through the cooking process (cooking media was confirmed to have concentrations of PFASs less than limits of detection prior to cooking commencing). All PFASs detected in the biota tissue samples were qualitatively compared, but only the dominant PFASs detected in biota tissue were selected for statistical comparison. Concentrations below LOD and LOQ were treated as 0.5 LOD and 0.5 LOQ respectively. The null hypothesis of no change in concentrations of PFASs was evaluated separately for different compounds, cooking treatments and species, by testing for parity (i.e. $\beta = 1$) between the concentrations in cooked and uncooked samples (using a Wald test). All models assumed an intercept of zero. All statistical analyses were undertaken using R v. 3.2.1 (R Core Team, 2016).

3. Results

3.1. General observations

PFASs detected in biota at levels greater than the limit of quantification included (Table S1) PFCAs (PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA), PFSAs (PFBS, PFHxS, PFOS, PFDS) and sulfonamide PFOS precursors (FOSA and NEtFOSE). PFASs were also detected in reference samples, as although there are no major point sources for PFASs in Wallis Lake, diffuse sources can impact the catchment. The dominant measurable PFASs included PFOS, PFHxS and PFOA, and these compounds are usually the drivers of dietary advice aimed at reducing PFAS exposure (e.g. Bhavsar et al., 2014; Williamtown Contamination Expert Panel, 2015). The following section focuses on statistical analyses for these dominant compounds, but other, non-dominant PFASs were also detected across the samples analysed both

Table 1
Summary of changes in non-dominant analytes for uncooked and cooked samples, showing frequency of detections where concentrations were above LOQ (Freq.), and mean analyte concentrations (Conc. [$\mu g\,kg^{-1}$], calculated for detects > LOQ, * is indicated where there were no detects). Symbols ↑, ↓ or – indicates an increase, decrease, or no change for the analyte (respectively) following cooking. For Dusky Flathead, two different cooking methods were used, so data for baked and fried samples are presented outside and inside brackets respectively.

Species	Sample type	Analyte	Uncooked		Cooked	
			Freq.	Conc. ($\mu g\,kg^{-1}$)	Freq.	Conc. ($\mu g\,kg^{-1}$)
School Prawn	Contaminated	PFNA	17%	0.16	100%↑	0.23↑
		PFDA	0%	*	67%↑	0.20↑
		PFHpA	0%	*	17%↑	0.19↑
		PFBS	0%	*	17%↑	0.09↑
Blue Swimmer Crab	Contaminated	PFHxA	50%	0.15	17%↓	0.10↓
		PFHpA	100%	0.21	83%↓	0.12↓
		PFUnDA	87%	0.11	0%↓	*↓
		PFDoDA	0%	*	17%↑	0.13↑
		FOSA	100%	0.31	100%–	0.32–
		NEtFOSE	33%	0.68	0%↓	*↓
	Reference	PFUnDA	17%	0.10	0%↓	*↓
		PFDoDA	17%	0.12	0%↓	*↓
		NEtFOSE	17%	0.89	0%↓	*↓
Dusky Flathead	Contaminated	PFDS	33%	0.14	33% (33%)–	0.38 (0.37)↑
		FOSA	0%	*	33% (33%)↑	0.20 (0.14)↑

before and after cooking (Table 1). Detection frequency and concentrations of non-dominant PFASs varied among species. PFCAs in School Prawn increased in detection frequency and concentration following cooking, whereas the converse was true for Blue Swimmer Crab (with the exception of PFDoDA; Table 1). PFSAs increased in detection frequency and concentration following cooking in both School Prawn (PFBS) and Dusky Flathead (PFDS). Sulfonamide PFOS precursors were detected in Blue Swimmer Crab and Dusky Flathead (Table 1). FOSA remained unchanged following cooking in Blue Swimmer Crab, but increased in detection frequency and concentration in Dusky Flathead. NEtFOSE decreased in detection frequency and concentration in Blue Swimmer Crab (Table 1).

3.2. Changes in concentrations of dominant PFAS analytes in biota following cooking

Changes in dominant PFAA concentrations following cooking were largely inconsistent among species and cooking treatments. PFOS, PFHxS and PFOA concentrations were dominant in School Prawn and Blue Swimmer Crab (Table S5). In School Prawn, uncooked concentrations of PFOS, PFHxS, PFOA were < 16.25 $\mu g\,kg^{-1}$, < 4.73 $\mu g\,kg^{-1}$ and < 0.31 $\mu g\,kg^{-1}$ respectively (Fig. 1). Overall, concentrations of PFOS, PFHxS and PFOA in School Prawn were significantly greater following cooking (Fig. 1, Table 2), with concentrations effectively doubling following boiling (Table 2). PFOS concentrations were < LOQ in ~83% of uncooked reference samples, but concentrations increased to > LOQ (mean 0.12 $\mu g\,kg^{-1}$) in 83% of samples following cooking.

Concentrations of PFOS, PFHxS and PFOA were lower in Blue Swimmer Crab than School Prawn, with uncooked concentrations < 6.45 $\mu g\,kg^{-1}$, < 0.85 $\mu g\,kg^{-1}$ and < 0.79 $\mu g\,kg^{-1}$ respectively. PFOS was measured in Blue Swimmer Crab at concentrations > LOQ in 83% of uncooked reference samples (mean 0.22 $\mu g\,kg^{-1}$), and PFOA was measured at concentrations > LOQ in ~33% of uncooked reference samples (mean 0.06 $\mu g\,kg^{-1}$). In contrast to School Prawn, there was no significant change in PFOS concentrations in Blue Swimmer Crab following cooking. However, PFHxS and PFOA concentrations were significantly lower following cooking, effectively halving in concentration following boiling (Fig. 1, Table 2).

PFOS and PFHxS were dominant PFASs in Dusky Flathead, with PFOA concentrations < LOQ in all samples. There was considerable variation in PFOS in uncooked Dusky Flathead, with concentrations ranging from < LOQ to 408 $\mu g\,kg^{-1}$, and low concentrations (mean 0.11 $\mu g\,kg^{-1}$) present in uncooked reference samples. All cooked and uncooked reference samples had PFHxS concentrations < LOD. Frying did not lead to a significant difference in PFOS concentrations, however baking led to a minor but significant increase in PFOS concentration (Fig. 2, Table 3). While PFHxS concentrations were much lower than PFOS, baking and frying both led to a significant concentration increase for this analyte.

A change in tissue mass could contribute to a change in contaminant concentration, particularly if water is lost from the tissue during cooking (decreasing tissue mass), without losing PFASs. Consequently, tissue mass was compared before and after cooking, and showed a significant decrease in all cooking treatments (Table 3, note that for boiling data was only available for School Prawn). The level of mass loss was relatively consistent (~15%) among cooking treatments (Fig. 3, Table 3).

3.3. Changes in PFAS concentrations in cooking media

In the residual water following cooking, several PFASs were detected > LOQ including PFCAs (PFHxA, PFHpA, PFOA, PFNA), PFSAs, (PFHxS, PFOS) and the PFOS precursor NEtFOSE. PFOA, PFOS and NEtFOSE were also detected in the water used to cook the reference samples. In the water used to boil the Blue Swimmer Crab from the contaminated site, PFOS (31–84 $ng\,L^{-1}$), PFHxS (38–92 $ng\,L^{-1}$) and PFOA (14–87 $ng\,L^{-1}$) were detected in 100% of the samples. In addition, PFHpA (1.3–103 $ng\,L^{-1}$), PFHxA (< 2.0–25 $ng\,L^{-1}$), PFDoDA (< 2.5–5.4 $ng\,L^{-1}$) and NEtFOSE (< LOD–26 $ng\,L^{-1}$) was found in 83%, 83%, 83% and 33% of the samples, respectively. PFOS, PFOA, and NEtFOSE were also detected in the cooking media for some Blue Swimmer Crab reference samples.

In the water used to boil School Prawn, PFOS (90–138 $ng\,L^{-1}$), PFHxS (107–283 $ng\,L^{-1}$), PFNA (< 2.5–3.8 $ng\,L^{-1}$), PFOA (5.5–15 $ng\,L^{-1}$), PFHpA (1.4–4.1 $ng\,L^{-1}$) and PFHxA (8.5–16 $ng\,L^{-1}$) were found in 100% of the samples. PFBS (< LOD–18 $ng\,L^{-1}$) was found in 66% of the samples, and PFOS and PFPeA were also detected in some reference samples. The amount of these substances found in the water indicates a percentage loss from the corresponding biota in the order; PFHpA > PFHxS > PFOA > PFOS, with PFHpA having the greatest relative loss from the biota to the cooking media. It is important to highlight that as School Prawn and Blue Swimmer Crab were boiled as whole organisms (as this is the standard way in which these species are cooked), PFAAs in the cooking media could have been liberated from other organs or body tissues.

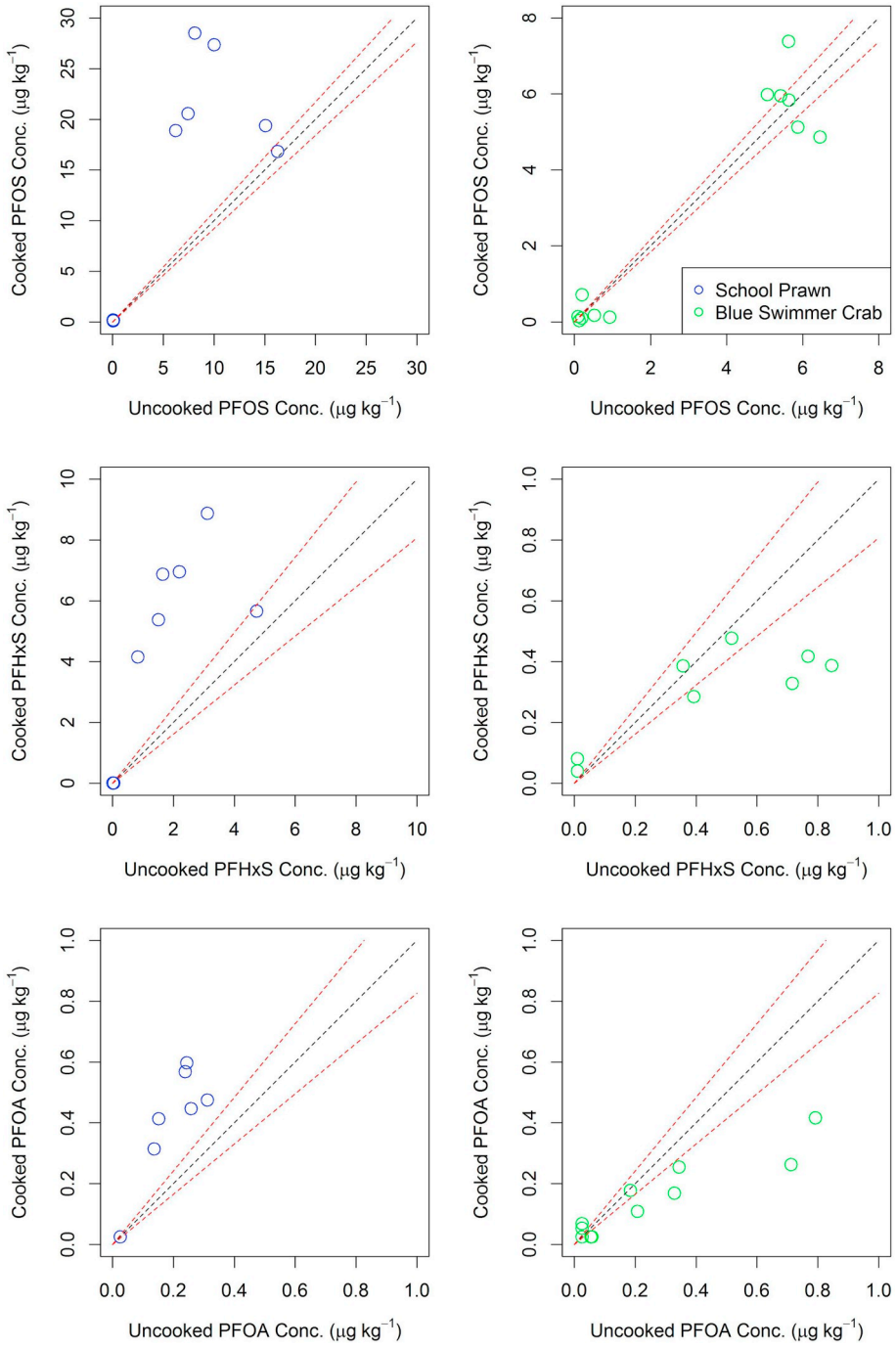

Fig. 1. Concentrations of PFOS (top panel), PFHxS (middle panel) and PFOA (lower panel), in uncooked and cooked (by boiling) School Prawn and Blue Swimmer Crab (see legend). The black dashed line represents the line of parity between the *x*- and *y*-axis, and the dashed red line represents analytical variance for each compound calculated from duplicate measurements of the same sample (variance for PFOS is used for PFHxS and PFOA, see Supplementary Information).

PFOS was the only PFAS detected > LOQ in residual oil following cooking, and was found in 42% of oil samples (mean 1.81 µg kg^{-1}), following the baking and frying of Dusky Flathead from the contaminated site. In the samples where PFOS was found, the total amount of PFOS represented an average loss (as a percentage of the amounts in the corresponding raw seafood) from the biota to the oil of 0.63%. No PFASs were detected in any residual oil sample following cooking of the reference samples.

4. Discussion

The results clearly demonstrate that the effect of cooking on concentrations of PFASs is inconsistent among species, cooking methods, and PFAS compounds. This study is significant in that it is the first PFAS cooking study to measure liberated PFAAs in the cooking media, and also the first such study to evaluate a crab species. Dominant analytes largely reflected the concentration ranges identified for these species in previous studies (Taylor et al., 2018; Taylor and Johnson, 2016), however this is the first report of sulfonamide precursors in Blue Swimmer Crab and Dusky Flathead. The biologically related crustacean species (i.e. School Prawn and Blue Swimmer Crab are both members of Decapoda) showed patterns that were largely opposing, despite an identical cooking approach being employed. Increases in PFHxS concentration following cooking were observed in

Table 2
Outcomes of statistical models evaluating concentrations of dominant PFAS analytes between uncooked and cooked samples.

Species	Cooking type	Analyte	β	F	P
School Prawn	Boiling	PFOS	1.70	6.24	0.029[a]
		PFHxS	2.18	12.55	0.004[a]
		PFOA	2.01	56.28	< 0.001[a]
Blue Swimmer Crab	Boiling	PFOS	1.10	3.25	0.099
		PFHxS	0.58	46.29	< 0.001[a]
		PFOA	0.50	139.98	< 0.001[a]
Dusky Flathead	Frying	PFOS	1.04	0.92	0.359
		PFHxS	1.12	12.92	0.005[a]
	Baking	PFOS	1.18	11.78	0.006[a]
		PFHxS	1.56	13.31	0.005[a]

[a] Indicates slope (β) is significantly different from 1.

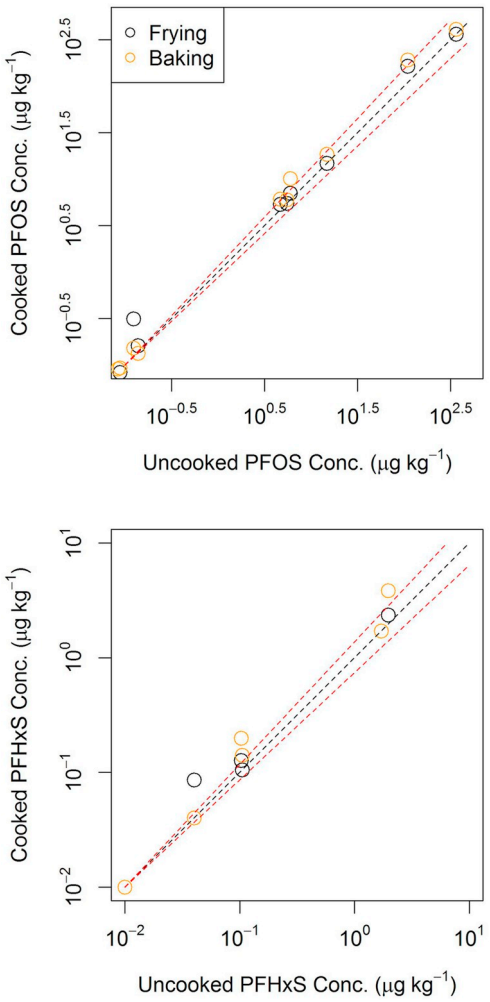

Fig. 2. Concentrations of PFOS (top panel) and PFHxS (lower panel), in fried and baked (see legend) Dusky Flathead. The black dashed line represents the line of parity between the *x*- and *y*-axis, and the red line represents analytical variance for each compound calculated from duplicate measurements of the same sample (variance for PFOS is used for PFHxS, see Supplementary Information).

both School Prawn and Dusky Flathead, which were treated to different cooking conditions (boiling, frying and baking). Patterns in the concentration and frequency of non-dominant PFASs showed similar patterns to PFHxS, with the majority of PFAA concentrations decreasing following cooking in Blue Swimmer Crab, and increasing following cooking in School Prawn and Dusky Flathead.

The outcomes of our experiments reiterate the contrasting findings

Table 3
Outcomes of statistical models evaluating changes in the mass (g) of uncooked and cooked samples.

Species	Cooking type	β	F	P
School Prawn	Boiling	0.86	14.98	0.003[a]
Dusky Flathead	Frying	0.83	307.31	< 0.001[a]
	Baking	0.84	15.61	0.003[a]

[a] Indicates slope (β) is significantly different from 1.

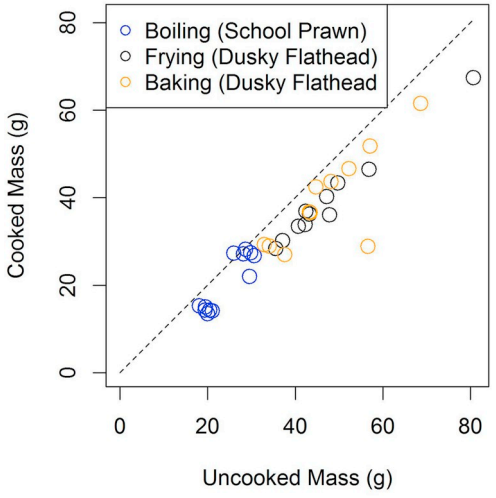

Fig. 3. Cooked and uncooked sample mass for boiling (School Prawn), frying (Dusky Flathead) and baking (Dusky Flathead) treatments. The black dashed line represents the line of parity between the *x*- and *y*-axis.

on the effects of cooking in the literature, which are outlined in the Introduction. These studies suggest various mechanisms to explain the patterns observed, including water loss, differing bioaccumulation of different PFASs among species, mass loss, heat-facilitated conversion of precursors, volatilisation, and chemical loss to cooking media (Bhavsar et al., 2014; Del Gobbo et al., 2008). The influence of some of these putative mechanisms in our study is discussed in detail below.

4.1. Effects of cooking on contaminant concentrations

Concentrations of both dominant and non-dominant PFAAs were all found to increase in School Prawn following boiling, and there are a multiple plausible mechanisms which could have contributed to this outcome. Niamnuy et al. (2007) showed that for White Shrimp *Penaeus indicus*, boiling for 4 min in a 2% salt solution led to an ~2% decrease in protein content, but a 32% decrease in moisture content. While moisture content of School Prawn was not measured in our experiment, we measured tissue mass loss of ~14% for School Prawn of which the majority was likely to be moisture. Vassiliadou et al. (2015) showed ~50% moisture loss in shrimp following frying, which is much greater than the mass loss measured from boiling. Assuming there were no major losses of protein during cooking in our study, decreases in water content would increase the ratio of protein in the cooked tissue and lead to overall higher wet-weight based PFAA concentrations (Weber et al., 2008). While this could contribute to the doubling of concentrations that was observed in School Prawn, overall mass loss was lower than 50% which suggests that other processes may have contributed.

The polar sulfonate and carboxylate functional groups, which are paired with the hydrophobic fluorinated carbon tail, result in a hydrophobic and ionic interaction with proteins that make PFAAs proteinophilic (Bischel et al., 2010; Han et al., 2003, 2005; Jones et al., 2003). Many metals also show proteinophilic properties, and have been found to show increased or unchanged concentrations in fish after

cooking (e.g. Hg, As, Hamza-Chaffai et al., 1995; Morgan et al., 1997; Perelló et al., 2008). While there were only minor changes in the total protein fraction in the White Shrimp study (Niamnuy et al., 2007), there was a dramatic increase in the proportion of denatured protein in cooked tissue (from 0% to ~62%). This was accompanied by equivalent decreases in myofibrillar and sarcoplasmic protein (serum proteins were not measured). These changes in protein structure may have affected the binding of PFAAs in the tissue which could have contributed to the changes observed.

In Blue Swimmer Crab, PFHxS, PFOA and non-dominant PFCAs had a lower concentration in boiled samples compared to the corresponding raw sample, indicating a decrease in concentration following boiling. PFOS, however, showed no change in concentration. A range of studies highlight changes in the tissue composition of crabs following cooking. Boiling of Edible Crab *Cancer pagrus* for 12 min in a 2% salt solution led to an increase in relative protein content by as much as ~5%, and a 6% decrease in moisture content (Maulvault et al., 2012). Similarly, Southern King Crab *Lithodes santolla* showed an increase in relative protein content of 3% (Risso and Carelli, 2012). Conversely, boiling of Blue Crab *Callinectes sapidus*, led to 3–4% protein loss (Hanover et al., 1973), and this cooking method led to a loss ~25% of PCB contaminants in this species, the majority of which partitioned into the cooking media (Zabik et al., 1992). The overall concentration of PFHxS in Blue Swimmer Crab was an order of magnitude lower than in School Prawn, and this may have made smaller losses more noticeable; however, PFOA concentrations were roughly equivalent between these species and a similar decrease to PFHxS was observed. Unfortunately, because of the method employed to prepare and cook the crabs, paired mass measurements could not be obtained to evaluate tissue mass loss through the experiment, and loss of a small amount of protein (with bound PFAAs) remains a possibility. PFAAs were detected in the cooking media for Blue Swimmer Crab which indicates some losses from the seafood occurring, but this would reflect losses from all body tissues, not just edible tissues. Nonetheless, PFASs in the cooking media could explain the concentration patterns observed, as PFHpA, PFOA and PFHxS had a greater relative loss to the water compared to PFOS. The shorter fluorocarbon chain-length of PFHxS (C6-fluorocarbon), as well as the carboxylate functional group of PFHpA (C6-fluorocarbon) and PFOA (C7-fluorocarbon), give these compounds a greater hydrophilic character compared to PFOS and thus making them more soluble in water (Kelly et al., 2009).

Similar factors to those listed above for School Prawn could explain the small increases in PFOS (for baking) and PFHxS concentrations (for both cooking treatments) in Dusky Flathead. Concentrations of PFAAs in residual oil suggested only small losses to the cooking media occurred. Sungur et al. (2019) suggest that heated cooking oil may act like an extraction solvent, thus explaining the high loss of PFAAs from fried fillets in their study; our results do not support this hypothesis. In our study, there is a possibility that the increased frequency of detection and concentration of PFAAs in cooked School Prawn and Dusky Flathead could have arisen through the heat-facilitated conversion of precursors, although Del Gobbo et al. (2008) suggest that there is little evidence available in the literature to support this. Any number of precursors could have been present in uncooked tissue, but our samples were only tested for a limited suite of PFAA precursors. Consequently, this remains a question to consider in future studies.

4.2. Technical considerations and implications for exposure risk

This study employed an improved experimental design and statistical approach to detect changes in PFAA concentrations following cooking relative to previous studies, however certain factors limited our interpretation. Firstly, the factors contributing to the contrasting patterns observed are unclear, and there are likely to be biochemical and physiological differences among the three species investigated. Supplementary testing of changes in protein, lipid and moisture content following cooking would aid in interpretation, and also assist in comparisons between different studies and species. Secondly, while all PFCAs and PFSAs were recovery-corrected from the internal standards, no internal standards were available for FOSA, NEtFOSAA, NMeFOSAA, NMeFOSE and NEtFOSE. Consequently, an external calibration was used and the resultant data are thus not recovery corrected and should be interpreted with caution. Finally, analysis of precursor compounds is necessary to effectively evaluate heat-facilitated conversion of precursors, and the influence of this process on dietary exposure risk associated with seafood consumption. Incorporation of a Total Oxidisable Precursor Assay (TOPA) would help identify these transitions through the cooking process, but this was beyond the scope of the current study.

The rationale underpinning this study was to establish whether conventional cooking methods led to decreases in PFAA concentrations in edible seafood. This question was of interest to assess if dietary advice, which is often provided to manage exposure to PFAAs through consumption of seafood, could be moderated (since most data used in exposure modelling are from uncooked samples). Unfortunately, the findings of Bhavsar et al. (2014), Vassiliadou et al. (2015) and the current study, largely confirm that cooking does not consistently moderate this exposure. The substantial differences in the results of these studies, and the patterns reported in Del Gobbo et al. (2008) and Sungur et al. (2019) remain unexplained. This highlights the need for future work, which may involve applying consistent experimental procedures across some of the species studied in these contrasting investigations, which would help resolve if the differences arise due to physiological or procedural differences. Despite the contrasting patterns in the literature, studies such as these still have a role in exposure assessments given that most food is consumed after some form of cooking or processing has occurred.

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Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.03.032.

Appendix A. Supplementary data

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