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Legacy and novel perfluoroalkyl substances in raw and cooked squids: Perspective from health risks and nutrient benefits

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ABSTRACT

Perfluoroalkyl substance (PFAS) existed ubiquitously in the environment and could be ingested unconsciously with food which posed a disease risk to human health. Swordtip squid (*Uroteuthis edulis*) is one of the most popular and highly consumed seafood worldwide, with wide distribution and abundant biomass. Therefore, it is of great importance to the health of the public by reducing the health risks of squid consumption while preserving the benefits of squid to humans. In this study, the PFAS and fatty acids in squids were tested from the southeast coastal regions of China, a major habitat for squids. Relative higher concentrations of PFAS in squid were found in the subtropical zone of southern China (mean: 15.90 ng/g·dw) compared to those of the temperate zone of northern China (mean: 11.77 ng/g·dw). The digestive system had high tissue/muscle ratio (TMR) values, and the pattern of TMR among the same carbon-chain PFAS was similar. Cooking methods have a significant contribution to eliminating PFAS (in squids). PFAS were transferred from squids to other mediums after cooking, so juice and oil should be poured out to minimize PFAS exposure into body. The result showed that squids can be regarded as a healthy food by health benefits associated with fatty acids. Estimated daily intake (EDI) had the highest level in Korea via consuming squids through cooking processes compared with other countries. Based on the assessment of the hazard ratios (HRs), there was a high exposure risk of perfluoropentanoic acid (PFPeA) via taking squids for human health. This research provided the theoretical guidance of aquatic product processing in improving nutrition and reducing harmful substances.

1. Introduction

Perfluoroalkyl substance (PFAS) contamination extends beyond planetary boundaries due to their high environmental persistence, widespread and continued use, and a range of potential impacts (Cousins et al., 2022). In recent decades, long-chain PFAS (defined as perfluoroalkyl sulfonic acids ($C_nF2_{n+1}SO_3H$, $n \geq 6$, PFSAs) and perfluoroalkyl carboxylic acids ($C_nF_{2n+1}COOH$, $n \geq 7$, PFCAs) have received increasing attention because of their properties of persistence, bioaccumulation, and possible toxicity to humans (Itoh et al., 2019), and hence the variety of restrictive actions have been taken to reduce the release of these compounds. Perfluorooctane sulfonic acid (PFOS), perfluorooctanoate acid (PFOA), and perfluorohexane sulfonic acid (PFHxS) have been designated as persistent organic pollutants (POPs) in the Stockholm Convention, and restricted their uses in industries (Stockholm Convention, 2017; UNEP, 2021). Relevant enterprises have shifted away from long-chain PFAS to short-chain or novel replacements, such as perfluoroalkyl ether carboxylic acids (PFECAs) and perfluoroalkyl ether sulfonic acids (PFESAs).

The occurrences of short-chain or novel contaminants have been reported in daily foods such as carps, eggs, grains, bullfrogs, vegetables, and fruits (Brown et al., 2020; Shi et al., 2015; Sun et al., 2021a). Dietary exposure is a significant PFAS exposure pathway for humans, particularly in aquaculture foods (Susmann et al., 2019). Seafood has been

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essential to our daily diet, providing an important part to meet the body's needs for amino acids, protein, vitamins, fatty acids, and minerals. Squids are regarded as the economic seafood with high-quality protein and nutrients, which are essential for maintaining well-balanced human physiological functions.

The squid *(Uroteuthis edulis)* was widely distributed throughout the world shown in Figure S1. Squid fishery has been developed in China since 1989, and has been continuously expanding the scale because squid is highly productive (Dong et al., 2020). Nowadays, the annual production of squids from China has been estimated to reach 1 million tons approximately. China has been regarded as the main producer, consumer, and exporter of squids in the world (Wei et al., 2016; Zhou, 2019) (Table S1). Our previous study evidenced great concentration and bioaccumulation of PFAS in squids from the South China Sea (Diao et al. 2022). Applying stable isotope ratios to analyze the trophic level and carbon source have shown that squids as a kind of classic aquatic organism played an essential role in marine ecosystems as predators and prey. Meanwhile, squids have a fast growth rate, short lifespan, and easier collection making them more suitable for monitoring marine environmental pollutants (Lischka et al., 2020; Wu et al., 2017).

Cooking can help outcomes of characteristic pleasant taste, which is accepted as a common method of consumption of squids, such as steaming, baking, and frying (Hu et al. 2020). Cooking method can be determining factor to influence the fatty acids in foods because of a series of chemical and physical reactions. Modified fatty acids can affect PFAS concentrations and compositions because these two substances were reported that had a coupling relationship (Yamada et al. 2014). Because PFAS has a hydrophilic head and hydrophobic tail that targets a fatty acid-binding protein (FABP), PFAS can compete with fatty acids. Therefore, further studies are needed to better understand the altered mechanisms between PFAS and fatty acids during cooking processes, including steaming, baking, and frying. Changing fatty acids could affect the health risk for humans. For example, saturated fatty acids (SFAs) can raise the blood cholesterol level, low-density lipoprotein cholesterol, and arterio-sclerosis risk (Sun et al. 2023). The balanceable proportions of atherogenicity index (AI), peroxidisability index (PI), and thrombogenicity index (TI) were reported to evaluate the risks of diseases (Abdel-Naeem et al., 2021; Zula et al., 2021).

Most of the studies focused on PFAS and fatty acids in the edible part of seafood in raw samples. Unpredictable variations can occur through cooking processes, which can also lead to changes in PFAS and fatty acids. How it affects human health is an important and hot issue. To bridge this knowledge gap, we investigated the concentrations and compositions of PFAS and fatty acids in raw and cooked squids in various tissues. The objectives of the present study were to: (1) analyze the variation and influence of PFAS in different sizes of squid at sampling sites; (2) compare the distribution patterns of individual PFAS in different tissues; (3) evaluate the changes of PFAS in squids according to various cooking methods; and (4) assess potential risk-benefits in PFAS and fatty acids for human of each country affected by cooking. The present study is the first report on health risks of PFAS and fatty acids via consuming squids through cooking processes, which can help better understand food safety and potential human health, and afford fundamental knowledge for risk assessments and management of emerging pollutants.

2. Materials and methods

2.1. Sample collection

Squids are a kind of economic seafood, mainly distributed in the tropical sea and temperate sea, surrounding Guangdong, Fujian, Zhejiang, and Shandong Province, China, which these areas have abundant coastal and marine resources. These areas also have intensive human disturbance and a high degree of industrialization, and environmental pollution in these sites has attracted consideration from the public. In this study, all squids were collected at local docks along the eastern coastal areas of 6 cities in China (Zhanjiang City, Shantou City, Quanzhou City, Zhoushan City, Lianyungang City, and Qingdao City, generally from south to north) from September to November 2021. To avoid the error of different species, *Uroteuthis edulis* was selected as the sole subject to study the differences in PFAS and fatty acids at various sites. The small-sized and large-sized squids were classified according to the previous research (Yamaguchi et al. 2019). The mantle length and weight for small-sized squids were 159.5 ± 13.3 mm and 109.8 ± 15.8 g ($n = 72$), and the mantle length and weight for large-sized squid were 330.8 ± 31.6 mm and 417.7 ± 84.1 g ($n = 36$). More related information on squids was shown in Table S2. Sampling locations and specific information are listed in Figure S2. All samples were stored in polypropylene (PP) containers and kept in iceboxes for transport. Then squid samples were immediately dissected, including tentacle, buccal mass, head, ink sac, gill, cartilage, muscle, digestive system, and fin stored in a refrigerator at −20 °C until treatment. Edible parts were cooked by steaming, baking, and frying immediately. Additional details of cooking methods were shown in *Supplementary Material*. All tools and containers were washed before use by rinsing sequentially with methanol and Milli-Q water to avoid analytical interference.

2.2. Materials and reagents

A total of twenty-two native standards of individual PFAS compounds and nine mass-labeled internal standards with purities of > 98% were bought from Wellington Laboratories (Guelph, Ontario, Canada) (The full names for all these compounds are listed in Table S3). All PFAS were divided into novel and legacy PFAS. Legacy PFAS were subdivided into long-chain and short-chain PFAS. The six novel PFAS includes 2,2,3,3-tetrafluoro-3-(trifluoromethoxy) propionic acid (PFMOPrA), perfluoro (4-methoxybutanoic) acid (PFMOBA), sodium dodecafluoro-3H-4,8-dioxanonanoate (ADONA), chlorinated polyfluorinated ether sulfonate (F-53B) and perfluoro-(2,5,8-trimethyl-3,6,9-trioxadodecanoic) acid (HFPO-TeA). Specific categories were presented in *Supplementary Material*. Chromatographic grade methanol (MeOH) (HPLC grade) and acetonitrile (ACN) (HPLC grade) were chosen from J. T. Baker (Phillipsburg, NJ, USA); chloroform, sodium chloride, sodium bicarbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3), ammonium hydroxide (NH_4OH) and sulfuric acid (H_2SO_4) from Xilong Science (Jinping District, Shantou, China); normal hexane (n-hexane) from Sigma-Aldrich Co (St. Louis, MO, USA). The analytical standards of fatty acids were from Shang Hai Yuan Ye Bio-Technology Co., Ltd (Qingpu District, Shanghai, China). Related information on individual PFAS, including chemical formula, parent ion, quantitative ions, and qualitative ions, are shown in Table S3.

2.3. Sample treatment of target analytes

All samples with internal standards (ISs) were extracted and concentrated under nitrogen gas. To reduce interference, the sample was purified by solid-phase extraction (SPE) with ENVI-Carb and Oasis WAX. Finally, eluents were concentrated under high-purity nitrogen for instrumental analysis. Detailed information on chemical extraction and cleanup for PFAS and fatty acids was shown in *Supplementary Material*. Detailed descriptions of the instrumental conditions and parameters were summarized in Table S4 and Table S5.

2.4. Cooking methods

The large squids were mixed and divided into different experimental groups to avoid individual differences. There were three copies in each experimental group to avoid experimental errors. Based on dietary habits in China, edible parts including muscles, fin, and tentacles of squid were cooked by three cooking methods in this study: steaming, baking, and frying (Hu et al., 2020). To reduce interference, all PTFE (polytetrafluoroethylene) materials were prohibited throughout the experiment. All tools were washed with Milli-Q water and methanol before cooking. Samples with equal mass subjected to each cooking method and the weight (raw and cooked samples) were recorded.

In the present study, steaming samples were put in a steamer on a medium fire and cooked for 8 min when ultrapure water was boiling. One liter of ultrapure water was boiled, repeating the above procedures as the control group. Frying samples were put into a stainless-steel frying pan when the oil was heated and fried with 100 g of oil preheated for 3 min. One hundred grams of oil was heated and repeated the above procedures as the control group. The roasting squids are wrapped in tin foil and baked in the oven for 15 min. Water and oil samples were extracted immediately; then biological samples were treated by vacuum freeze-drying, grinding, and extraction.

2.5. Statistical analysis

All analyses were processed by OriginPro 9.0 (OriginLab Corporation, USA), ArcGIS V10.2 software (ESRI, Redland, CA, USA), Excel 2016 (Microsoft Corporation, USA), and SPSS (SPSS Inc. Quarry Bay, HK). The data sets did not meet the criterion of homogeneity of variances, so the non-parametric statistical analysis was used. Differences between sites and sizes of squids were analyzed by the Mann-Whitney *U* test. Meanwhile, the differences of the PFAS concentrations between raw squids and various cooked squids were tested by the Mann-Whitney *U* test. The analysis in legacy and novel PFAS were tested by Kruskal-Wallis H with a statistical significance threshold of p less than 0.05 to compare the differences in concentrations.

2.6. Quality assurance

Before taking tests in all samples, polytetrafluoroethylene or other fluoropolymer materials were replaced with other materials to control strictly background pollution. All tools and containers were rinsed with methanol and Milli-Q water before use. Procedural blanks were analyzed every 12 samples to avoid interference from the background. Solvent blanks were processed to make the data reliable for all experimental procedures. The blank experiment of PP containers was tested and all individual PFAS were not detected or below the detectable limit in procedural and solvent blanks. Experiments of matrix spikes were done with an internal standard method. More information on quality control were shown in *Supplementary Material*. Results in procedural blanks, limit of detection (LOD), and limit of quantification (LOQ) are summarized in Table S6.

The previous research reported interference such as fatty acids can affect PFBA in biological samples due to their limited fragmentation and small size (Bangma et al. 2021). Therefore, the confirmatory experiment for the primary transition of saturated oxo-fatty acid (SOFA) 3-oxo-dodecanoic acid (213 ➔ 59) was tested to verify the possible interference for PFBA and its internal standard ($^{13}C_4$ PFBA). The results of this study showed no interference signals at the same retention time as the detection of PFBA in biological samples and standards (Figure S3, S4). Some researchers did the same confirmatory experiment of potential interference for PFBA, and they also had not found other misidentifying signals at the same retention time as PFBA (Jia et al. 2022; Huang et al. 2022).

2.7. Health and risk assessment

The novel and legacy PFAS distributional patterns in the muscle and other tissues are calculated by individual tissue/muscle ratios (TMRs) to investigate the distribution characteristics of different tissues for individual PFAS (Robuck et al., 2021). The calculation is as follows:

$$TMR = \frac{Ctissue}{Cmuscle} \tag{1}$$

AI is determined according to the ratio of SFAs and unsaturated fatty acids in connection with the cardiovascular disorder. AI value is used to prevent the appearance of micro-and macrocoronary disease (Abdel-Naeem et al. 2021). The calculation is as follows:

$$AI = \frac{C12:0 + (C14:0 \times 4) + C16:0}{\Sigma MUFA + \Sigma PUFAn-3 + \Sigma PUFAn-6} \tag{2}$$

TI shows the risks of forming clots in blood vessels (Ulbricht and Southgate 1991; Sun et al. 2023). The calculation was as follows:

$$\mathrm{TI} = \frac{\mathrm{C14:0 + C16:0 + C18:0}}{0.5^{*}\Sigma\,\mathrm{MUFA} + 0.5^{*}\mathrm{PUFA(n-6)} + 3^{*}\mathrm{PUFA(n-3)} + \mathrm{PUFA(n-3)/PUFA(n-6)}} \tag{3}$$

The nutritional value index (NVI) is to evaluate the nutrition of fatty acids (Abdel-Naeem et al. 2021; Werenska et al. 2021). The calculation was as follows:

$$\mathrm{NVI} = \frac{\mathrm{C\,18:0 + C18:1}}{\mathrm{C\,16:0}} \tag{4}$$

PI is used to assess the correlation between the fatty acid composition of samples and susceptibility to oxidation (Werenska et al. 2021). The calculation was as follows:

$$PI = (monoenoic\ acid \times 0.025) + (dienoic\ acid \times 1) + (trienoic\ acid \times 2) + (tetraenoic\ acid \times 4) + (pentaenoic\ acid \times 6) + (hexaenoicacid \times 8) \tag{5}$$

The estimated dietary intake (EDI) was used to assess human exposure of PFAS via consumption of squid (Diao et al. 2022). The calculation was as follows:

$$EDI = \frac{C \times IR}{BW} \tag{6}$$

where C is the PFAS concentration in squids (ng/g dw), IR is the mean daily intake of suqids (g/day), and BW is the body weight of a person (kg).

The hazard ratio (HR) was used to analyze the health risks based on established reference doses (RfD). The calculation was as follows:

$$HR = \frac{EDI}{RfD} \tag{7}$$

HR of 1 or above poses a high risk to public health, while conversely, HR less than 1 shows a low risk (Sun et al. 2021a).

3. Results and discussion

3.1. Variation and influence of PFAS occurrences in squids

Dry weight-based concentrations of PFAS were analyzed for the benefit of comprehending and unifying distribution patterns. Relationships between PFAS concentrations and locations were analyzed, as shown in Fig. 1. Concentrations of PFAS in samples varied significantly

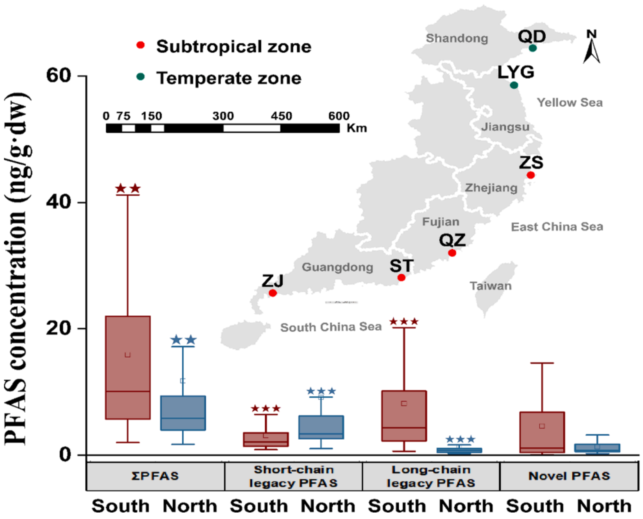

Fig. 1. PFAS concentrations at different sampling locations (ng/g·dw). **Note:** ZJ, ST, QZ, ZS, LYG, and QD represent Zhanjiang City, Shantou City, Quanzhou City, Zhoushan City, Lianyungang City, and Qingdao City, respectively. ★ represents the degree of significant difference. ★★★ means p is less than 0.001; ★★ shows p is less than 0.01; ★ describes p is less than 0.05.

among different sampling locations, with higher PFAS concentrations in the subtropical zone of southern China compared to the temperate zone of northern China (Mann-Whitney U test, $p < 0.05$). PFAS concentrations were detected in the subtropical zone, including Zhanjiang City, Shantou City, Quanzhou City, and Zhoushan City, which were higher than in the temperate zone, including Lianyungang City and Qingdao City.

In a previous study, it was reported that total PFAS concentrations in the environment have a significant effect on bioaccumulation (Zhang et al. 2013). Our previous studies have found that PFASs distributions and levels in red seaweed, *Porphyra haitanensis* had an analogous pattern in seawater (Sun et al., 2021b). Organisms can be exposed to PFAS directly or indirectly through respiration, ingestion, and skin contact. Because PFAS have persistent and bioaccumulative properties, organisms continue to accumulate long-half-life PFAS in the surrounding environment. The legacy PFAS also showed significant differences in the subtropical zone of southern China and the temperate zone of northern China (Mann-Whitney U test, p less than 0.05). Shantou City lies at the mouth of the Hanjiang River, Rongjiang River, and Lianjiang River. Quanzhou City situates in the hill terrain with well-developed river systems, and the estuary of Luojiang River and Jinjiang River. Zhoushan City is located in Hangzhou Bay, the estuary of Qiantang River. The above cities are located near the downstream of the river, while Lianyungang City and Qingdao City are not. The high concentration of PFAS in the subtropical zone may be because those sampling points are located downstream of the river and may become the sink of pollutants.

Various body sizes (and ages) of organisms showed differences in the uptake kinetics of contaminants after increasing the level of POPs in the environment. Great concentrations of PFAS were detected in small squids ranging from 2.00 to 154.11 ng/g·dw (mean: 17.98 ng/g·dw), while lower PFAS concentrations in the range of 2.00 to 47.97 ng/g·dw (mean:11.07 ng/g·dw) in large squids (Table 1). There was a similar result that significant negative correlations were found among different sizes with PFAS concentrations in alligators (Wang et al. 2013a). In this study, individual PFAS were analyzed in the estimated size of 108 samples, and a correlation was found between long-chain legacy PFAS and the sizes of squids shown in Figure S5 (Mann-Whitney U test, p less than 0.05). Among the long-chain legacy PFAS, the concentration in small squids (from 0.35 to 54.39 ng/g·dw) was also apparently higher than in large squids (from 0. 24 to 34.38 ng/g·dw). Studies have shown that age-concentration has a negative relationship that PFAS concentrations reduce considerably with increased age, according to regression

Table 1
PFAS concentrations in different individuals and sites of squids (ng/g·dw).

Items	**Large**						**Small**					
	Subtropical zone			**Temperate zone**			**Subtropical zone**			**Temperate zone**		
	Zhanjiang	Shantou	Quanzhou	Zhoushan	Lianyungang	Qingdao	Zhanjiang	Shantou	Quanzhou	Zhoushan	Lianyungang	Qingdao
Short-chain legacy PFAS	1.32–6.43 (3.17)	0.99–11.19 (3.47)	0.91–7.35 (2.68)	0.91–7.35 (2.68)	1.64–12.33 (5.28)	1.04–12.84 (3.89)	1.64–20.26 (5.70)	0.87–3.24 (1.66)	1.31–4.32 (2.58)	1.15–6.45 (2.61)	2.48–147.20 (22.15)	1.43–18.64 (5.25)
Long-chain legacy PFAS	1.01–4.20 (2.28)	1.70–34.28 (7.82)	2.05–12.38 (4.87)	1.32–15.50 (4.94)	0.29–1.14 (0.61)	0.24–12.26 (2.10)	4.12–35.02 (15.28)	2.49–19.22 (7.98)	5.71–54.39 (19.20)	0.62–10.87 (3.08)	0.35–3.72 (1.08)	0.42–5.85 (1.54)
Novel PFAS	4.70–21.67 (9.21)	0.21–22.16 (3.13)	0.16–27.03 (5.46)	0.24–10.23 (1.83)	0.41–4.11 (1.46)	0.26–1.12 (1.18)	1.30–26.98 (12.60)	0.36–1.88 (0.83)	0.32–11.99 (2.59)	0.10–5.80 (1.21)	0.44–3.19 (1.27)	0.34–2.96 (1.29)
ΣPFAS	9.22–30.23 (14.66)	3.28–47.97 (14.41)	3.66–35.90 (13.01)	4.75–19.06 (9.79)	2.74–17.15 (7.35)	2.00–19.71 (7.17)	22.42–50.49 (33.57)	4.98–21.52 (10.48)	7.70–59.71 (24.37)	2.00–18.58 (6.90)	3.55–154.11 (24.50)	3.84–26.50 (8.08)
PFAS	**2.00–47.97 (11.07)**						**2.00–154.11 (17.98)**					

analysis (Baduel et al. 2014).

The effect of size is somewhat counter-intuitive that PFAS should have higher levels in large biotas due to their persistence, refractory, and bioaccumulative characteristics. In previous research, Babut et al. (2017) also found a negative correlation between perfluorononanoic acid (PFNA) and PFOS content according to size or age in three cyprinid fish species (*Barbus barbus*, *Gobio gobio,* and *Rutilus rutilus*). There are several possible reasons why small individuals displayed significantly higher levels than large ones, as follows. Different absorption kinetics were shown in different sizes of biotas. Juveniles, such as rainbow trout, can not have an active mode of PFAS elimination, and PFAS concentrations are increased by enterohepatic recirculation and assimilation efficiency (Martin et al., 2003). In addition, the reproduction may be linked to reduced PFAS in larger squids (Peng et al., 2010). Of interest, Bangma et al. (2022) documented that reproduction was the crucial mode of maternal redistribution of PFAS to egg, newborn, or fetus in wildlife.

3.2. *Tissue-specific distributions of PFAS in squids*

PFAS were 100% detectable in all tissues with concentrations of 1.70–154.11 ng/g·dw (Fig. 2). PFAS had the highest accumulation in the digestive system of large and small squids. The mean concentration of PFAS in the digestive system of squids was 35.01 ng/g·dw. Findings built on similar results in previous research that the highest concentrations of PFAS were found in the digestive system among various tissues of bullfrog, crucian carp, and green eel goby (Hong et al., 2015; Sun et al., 2021a; Wu et al., 2019). Although gills were not at the highest PFAS concentrations, they showed high levels of PFAS (from 2.00 to 41.18 ng/g·dw) because gills are tissues of water exchange and can be directly exposed to contaminants in water.

PFBA was the predominant PFAS detected in samples. Distributions of PFAS in squids revealed interesting trends within the selected tissues that high concentrations of short-chain PFAS, such as PFBA, were found in different tissues of large and small squids. A comparison of predominant PFAS with our previous study revealed different patterns that PFOS and perfluoroheptanoic acid (PFHpA) were the dominant PFAS in *Porphyra haitanensis* and *Siganus fuscescen,* respectively (Sun et al., 2021b). This phenomenon seemed to be due to the spatial variations and discharge capacities of individual PFAS by surrounding industry, different trophic levels of biotas, and unique properties of compounds.

There were obvious differences in novel PFAS and long-chain legacy PFAS by Kruskal-Wallis H (p less than 0.05) (Figure S6). HFPO-TeA, a novel PFAS replacing PFOA, was found in squid tissues at detectable levels (detection rate 99%) and had a mean concentration of 3.00 ng/g·dw in all tissues (Figure S7). HFPO-TeA accounted for a higher proportion in various tissues of squids consistent with PFOA. This was due to ester bonds that could enlarge the molecular size and increase hydrophobicity. In addition, other novel PFAS, such as PFMOPrA, PFMOBA, sodium dodecafluoro-3H-4,8-dioxanonanoate (ADONA), and F-53B were detected to varying degrees as shown in Figure S8. Short-chain legacy and novel PFAS were at high levels, as perfluorinated-related industries in China had already begun to gradually replace the legacy PFAS (Wang et al. 2013b). In this study area, short-chain PFAS such as PFBA had a high detection rate and concentration in environmental samples (Diao et al., 2022; Sun et al., 2021a), thus high levels of PFBA could accumulate into biota.

The tissue distribution of contaminants determines fate of PFAS distribution and helps to understand the fundamental mechanisms in bioaccumulation better. TMR facilitates the assessment of contaminant partitioning between different tissues (Robuck et al., 2021). Contaminants that receive similar exposure and distribution mechanisms through all types of tissue are likely to have similar proportions across different tissues and vice versa. In the present study, TMRs for novel PFAS were presented and compared with those for legacy PFAS (Fig. 3), of which purpose was to explore the distribution of tissue and edible parts of squids. Similar distribution patterns were found for PFOS vs. chlorinated polyfluorinated ether sulfonate (F-53B), 2,2,3,3-tetrafluoro-3-(trifluoromethoxy) propionic acid (PFMOPrA) vs. PFBA, and perfluoro (4-methoxybutanoic) acid (PFMOBA) vs. perfluoropentanoic acid (PFPeA), suggesting that compounds of the same carbon chain share a similar distribution mechanism. The tissue distribution of individual PFAS varies from tissue to tissue, which may arise due to the biochemical property, structure, protein, and phospholipid content in

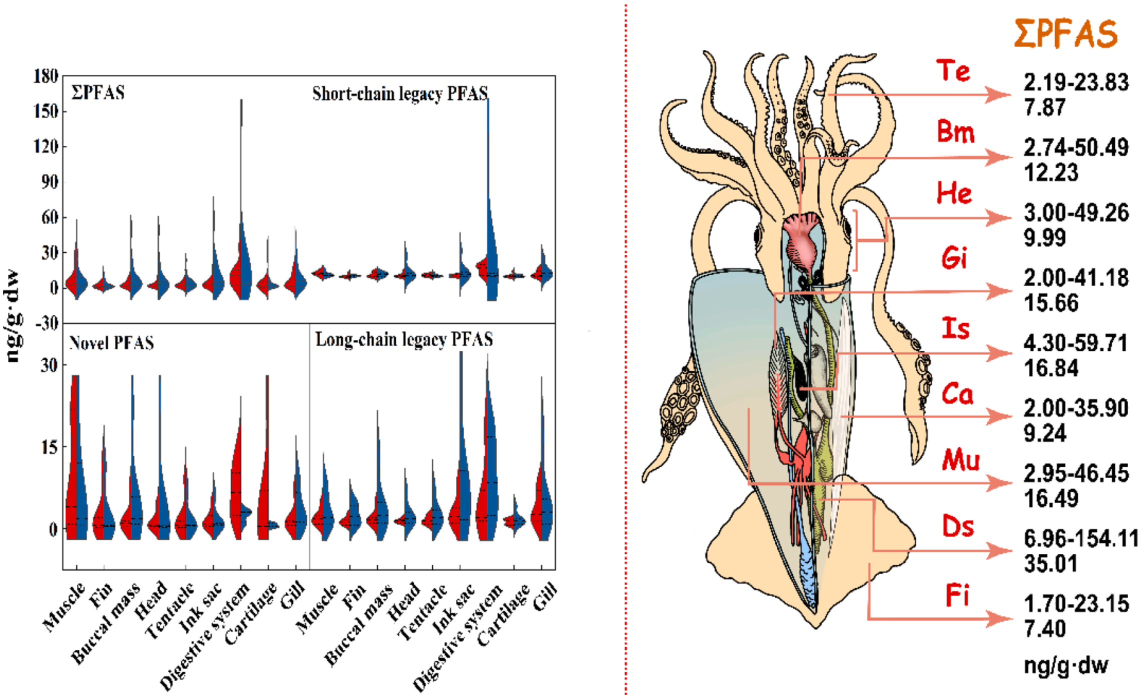

Fig. 2. PFAS concentrations in different tissues of squids (ng/g·dw). **Note: Te, Bm, He, Gi, Is, Ca, Mu, Ds, and Fi mean tentacle, buccal mass, head, gill, ink sac, cartilage, muscle, digestive system, and fin respectively.**

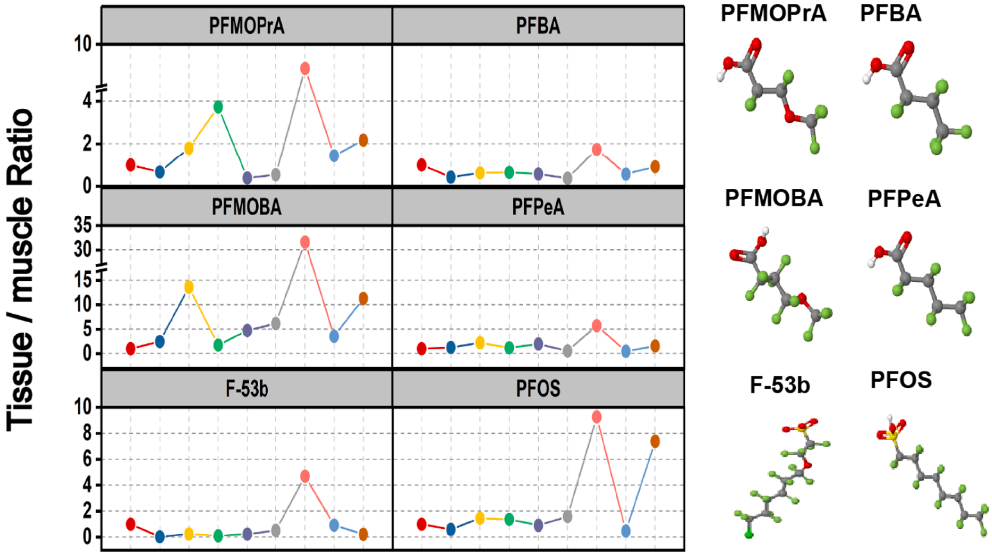

Fig. 3. TMR values of novel PFAS in tissues. **Note: TMR was the ratio of concentration in edible muscle parts and other individual tissue.**

tissues. Thus, the uptake and sorption of individual PFAS to different tissues may show slight variation even within the same species (Shi et al., 2015).

3.3. *PFAS changes after different cooking treatments*

The concentrations and compositions of PFAS in squids were investigated by various cooking methods. PFAS were detected in squids at concentrations ranging from 22.7 to 32.7 ng/g·dw (Fig. 4). The PFAS concentrations in squids were all reduced by the cooking method. This is a similar pattern to a previous study that reported PFAS concentrations reduced in cooked seafood (Vassiliadou et al., 2015). In this study, the most effective cooking methods to lower PFAS concentrations were baking, followed by frying and steaming. The concentration of PFAS was reduced by 30% in the baking method. Abdel-Naeem et al. (2021) reported that baking was the best cooking method to destroy muscle fibers and connective tissue in cooked samples, with good health benefits and consumer acceptance (Abdel-Naeem et al., 2021).

Mechanisms by which cooking can reduce PFAS concentrations are as follows. The protein-aggregated PFAS were destroyed at high temperatures and PFAS were transformed into a cooking medium. The blank juice and oil were treated by the same cooking process including volume of water, temperature, and cooking tools, but squids were not added. The concentration of PFAS in oil increased by 8% compared to the blank group (Table S7). PFAS can migrate from squids to oil which is the reason the concentration of PFAS in squid can be lowered by frying. The concentration of PFAS decreased after various cooking methods even though PFAS have good stability in thermal and chemical changes.

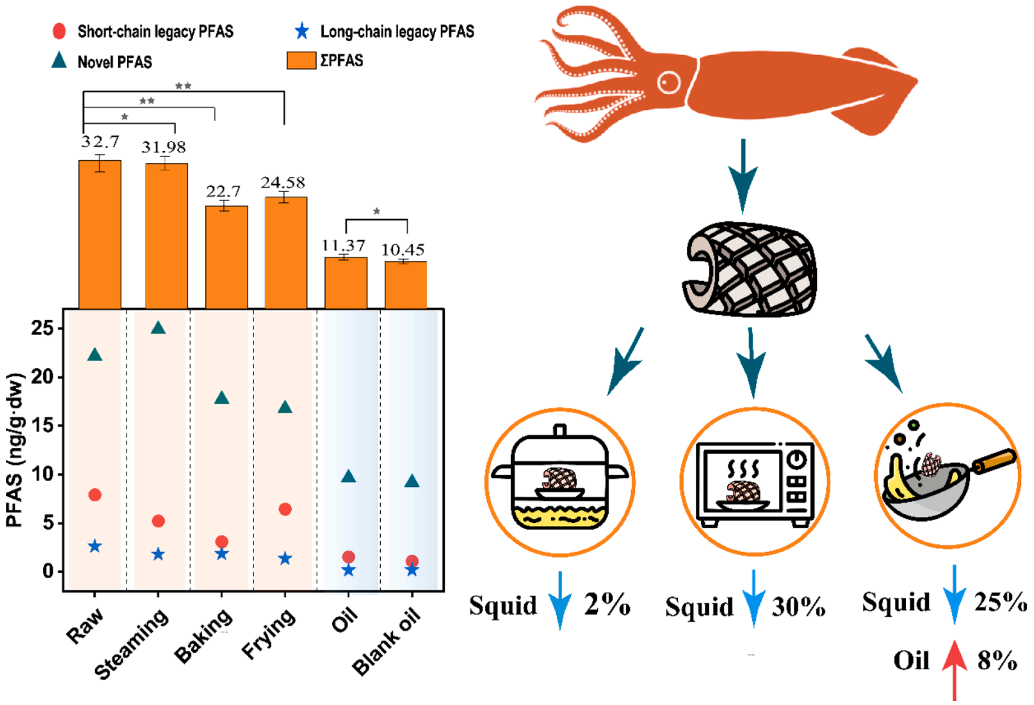

Fig. 4. PFAS in squids and oil by different cooking methods. Note: ★ represents the degree of significant difference. ★★★ means p is less than 0.001; ★★ shows p is less than 0.01; ★ describes p is less than 0.05.

Significantly, novel PFAS, as alternatives to typical PFAS, showed high detection rates reaching 100% in cooked samples, even though cooking reduced the levels of novel PFAS.

Seafood is now an important pathway to expose PFAS to humans in daily life. Raw squids, in comparison to other cooking samples, pose higher risks of PFAS exposure to the body for impacting immune function, neurodevelopment, and metabolic outcomes. Overall, the above descriptions are recommended highly for people who prefer raw squids for their delicious taste and nutritional value that ought to keep a watchful eye on potential health and food safety issues. Aquatic products contain high protein, fatty acids, and amino acids, which are good sources of dietary supplements. Nevertheless, we should focus on food safety and select pollution-free or healthy cooking methods to eliminate pollution.

3.4. Health benefits associated with fatty acids at different sites

Fatty acids in large and small squids were detected at different sites, as shown in Fig. 5. Squids with various body lengths were analyzed in this study and exhibited different fatty acid levels and nutritional values at different sites. Aquatic products seem to confer widespread advantages with items such as high protein, microelements, and essential fatty acids (Lund, 2013). But high fatty acids do not mean healthier. For example, SFAs appear to increase blood cholesterol levels and low-density lipoprotein cholesterol, which may improve arterio-sclerosis risk (Zula et al., 2021). ΣSFAs of large squid from other sampling sites showed higher values than monounsaturated fatty acids (MUFAs) except for Lianyungang City, whereas it was lower than polyunsaturated fatty acids (PUFAs).

ΣPUFAs/ΣSFAs and ΣPUFA (n-6)/ΣPUFA (n-3), as accepted parameters, have been applied to assess the healthiness and nutritional value of food for human consumption (Chen and Liu, 2020). ΣPUFAs/ΣSFAs proportion of over 0.4 in food was regarded as good food for human health to lower risks of cancer and cardiovascular diseases by the United Kingdom Department of Health (Werenska et al. 2021). In this study, it resulted from the high ΣPUFAs/ΣSFAs ratios in all samples of squid tissues ranging from 3 to 13, mainly depending on the sizes and sites (Table S8). Of note, all values of ΣPUFAs/ΣSFAs were above 0.4, which implied that squid, as appropriate seafood, does not cause disease in humans by increasing blood cholesterol.

ΣPUFA (n-3) and ΣPUFA (n-6) were found to be the predominant fatty acids, as expected. ΣPUFA accounted for 60%-78% of the total fatty acids shown in Table S8, consistent with the results reported in the previous study (Biandolino et al., 2021). ΣPUFA (n-6) is a pro-inflammatory factor, whereas ΣPUFA (n-3) is an anti-inflammatory factor (Harris et al., 2021). Hence, a suitable ΣPUFA (n-6)/ΣPUFA (n-3) value is of great significance to health for humans, reported that it can reduce the disease risk and incidence rate of coronary artery disease, asthma, diabetes, and rheumatic arthritis (Chen and Liu 2020). A suitable ΣPUFA (n-6)/ΣPUFA (n-3) values are 2.5 to 8.1 as recommended by the Food and Agriculture Organization of the United Nations (FAO); 4 to 6:1 accorded to Dietary Nutrient Reference Intakes of Chinese Residents (Abdel-Naeem et al., 2021; Duan et al., 2014; Kris-Etherton et al., 2000; WHO/FAO, 2003). All ΣPUFA (n-6)/ΣPUFA (n-3) values obtained in various squids were nearly within the recommended limit except that resulted from the small squids in Qingdao City. Mean value of small and large squids was 5.35 in different sites, which was in the range of adequate value for recommendations, which indicated squids were fit seafood for human health in this perspective of ΣPUFA (n-6)/ ΣPUFA (n-3).

AI and TI are associated with the regulation of glucose metabolism through food intake, stimulation of platelet aggregation, decreased blood pressure and serum triacylglycerol levels, and potential risk for coronary heart diseases (Chen and Liu 2020). There is also provided a target for the nutritional quality of fatty acids, where low quantifiable indicators represent food with better health and nutritional quality of

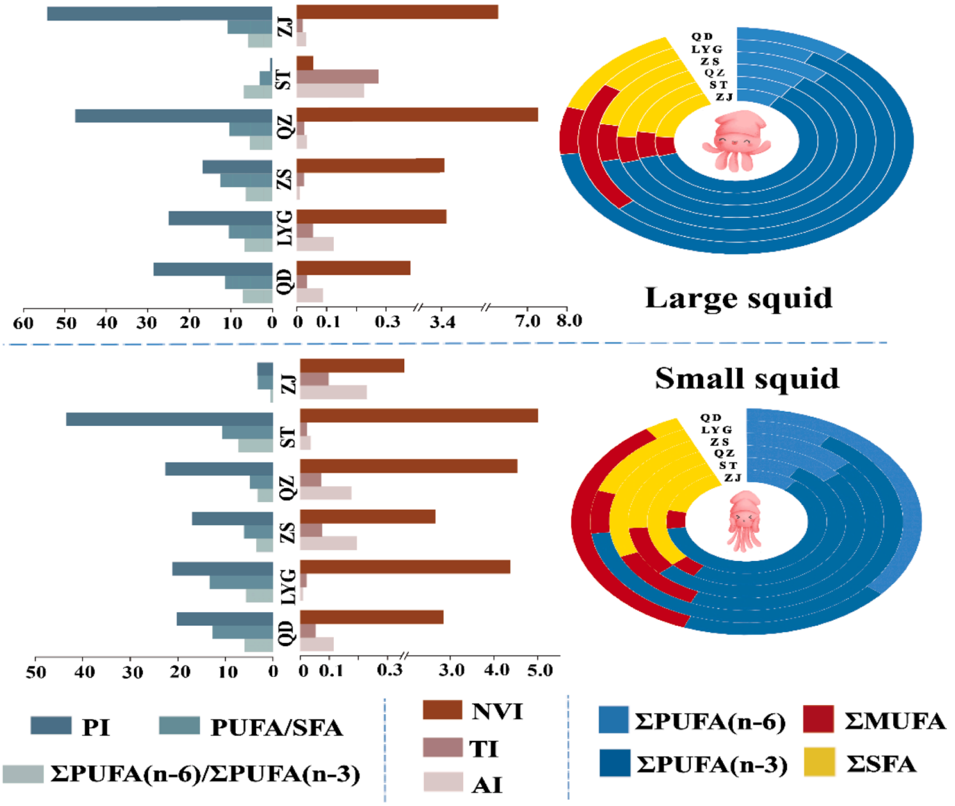

Fig. 5. The index of fatty acids levels at different sites. Note: ZJ, ST, QZ, ZS, LYG, and QD represent Zhanjiang City, Shantou City, Quanzhou City, Zhoushan City, Lianyungang City, and Qingdao City, respectively.

fatty acids and, subsequently, better protection from coronary diseases. AI and TI values of large squids were higher in Qingdao City than in other cities, which meant higher disease hazards in angiocardiopathy. In brief, variation trends of AI (from 0.01 to 0.23) and TI (from 0.02 to 0.27) were shown in all squids (Fig. 5; Table S8), and the values are relatively low than other research reported by Linhartová et al. (2018) who identified these values for *Oreochromis niloticus* (AI 0.63 and TI 0.61), *Clarias gariepinus* (AI 0.44 and TI 0.45) and *Oncorhynchus mykiss* (AI 0.37 and TI 0.41) (Linhartová et al. 2018). In this context, the small and large squids in different sites would indicate a high cardio-protective effect and low incidence of leading coronary heart diseases.

PI values were calculated based on the data given for squids in different sites between compositions of fatty acids and their susceptibility to oxidation. This index was applied to evaluate the stability of ΣPUFAs covering food and to protect from probable oxidation processes, so the greater PI value meant a higher protective potential for coronary artery disease (Werenska et al., 2021). All PI values in various squids had high levels except that resulted from the small squids in Qingdao City and the large squids in Lianyungang City; which mean value of small and large squids was 25.04 in different sites. Moreover, there were reported that rabbit, beef, chicken, and pork revealed lower sensitivity to lipid oxidation compared to fish, shrimp, and crab (Mapiye et al., 2011; Woloszyn et al., 2020), which indicated seafood had a higher protective potential for heart diseases. These data suggest that squid is a more suitable product for healthy nutrition for humans in suitable indicator of ΣPUFAs/ΣSFAs, ΣPUFA (n-6)/ΣPUFA (n-3), AI, TI, and PI.

3.5. Health risks and benefits of fatty acids influenced by cooking

Dietary fatty acids are crucial nutrition for many significant functions; they are a source of energy for numerous processes in the body, and support cell growth (Biandolino et al., 2021). After cooking processes, fatty acids may change their structure and content, affecting the quality of fatty acids in food. For example, the proportion of ΣSFAs, ΣMUFAs, and ΣPUFAs can fluctuate significantly after various processes. Although a large number of ΣMUFAs and ΣSFAs were investigated, ΣPUFAs were the primary fraction in all cooking treatments (Fig. 6). Compared with different processes, the proportions of ΣSFAs concentrations increased, and ΣPUFAs showed the opposite decreasing effect in all cooking methods, showing similar results in previous studies (Sun et al. 2023). The significant decrease in ΣSFAs and growth of ΣPUFAs from raw to cooked samples was explained by Uran for cooked Engraulis encrasicholus (Uran and Gokoglu, 2014) and by Biandolino for cooked Mytilus galloprovincialis (Biandolino et al., 2021). This is because ΣPUFAs can be converted to ΣSFAs, and ΣPUFAs have a higher melting point than ΣSFAs, so ΣPUFAs have a stronger tendency for degradation than ΣSFAs in the samples (Larsen et al. 2010).

The cooking method that most influenced the fatty acids in squids was the frying process in this study, which expressed a major variation when compared to various processes. This has been caused by the absorption in oil by the frying method, with the variation indicating individual fatty acid contents of the frying oil and oxidation of fatty acids (Biandolino et al., 2021). Based on these phenomena about fatty acids, various cooking processes will be analyzed separately. The ΣSFAs content of squids in frying was 11%, lower than in steaming (30%) and baking (33%) shown in Table S9, of which value is linked with cardiovascular disease, generating value-added of the low-density lipoprotein cholesterol. A decline in the ΣSFAs intake has been suggested to reduce the risk for disease (Strazdiņa et al. 2013).

The ΣPUFAs/ΣSFAs proportion, as a kind of indicator to assess the risk for cancer and cardiovascular diseases, was all higher than recommended value by different cooking methods. ΣPUFAs/ΣSFAs value in frying squids was 13.90, showing a higher level than raw and other processed squids, which identified the reality that the frying squids, in contrast to steaming and baking squids, represented to be healthier seafood, especially for humans subjected to cardiovascular diseases. The dietary significance of the ΣPUFA (n-6)/ ΣPUFA (n-3) by different cooking methods has long been known, and several studies reported that a balanceable proportion would contribute to the prevention and treatment of diseases (Biandolino et al., 2021). ΣPUFA (n-6)/ΣPUFA (n-3) in frying was 6.61 within the recommended limit; nevertheless, the values in steaming (0.31) and baking (0.27) were lower than the optimum values for human diets. It was caused by the high percentage of ΣPUFA (n-3), and the low proportion of ΣPUFA (n-6) in investigated steaming and baking samples.

Because seafood is considered a major source of ΣPUFAs, humans can cater for essential fatty acids via intake of seafood, particularly of the n-3 series eicosapentaenoic acid (EPA) and docosahexaenoic acid

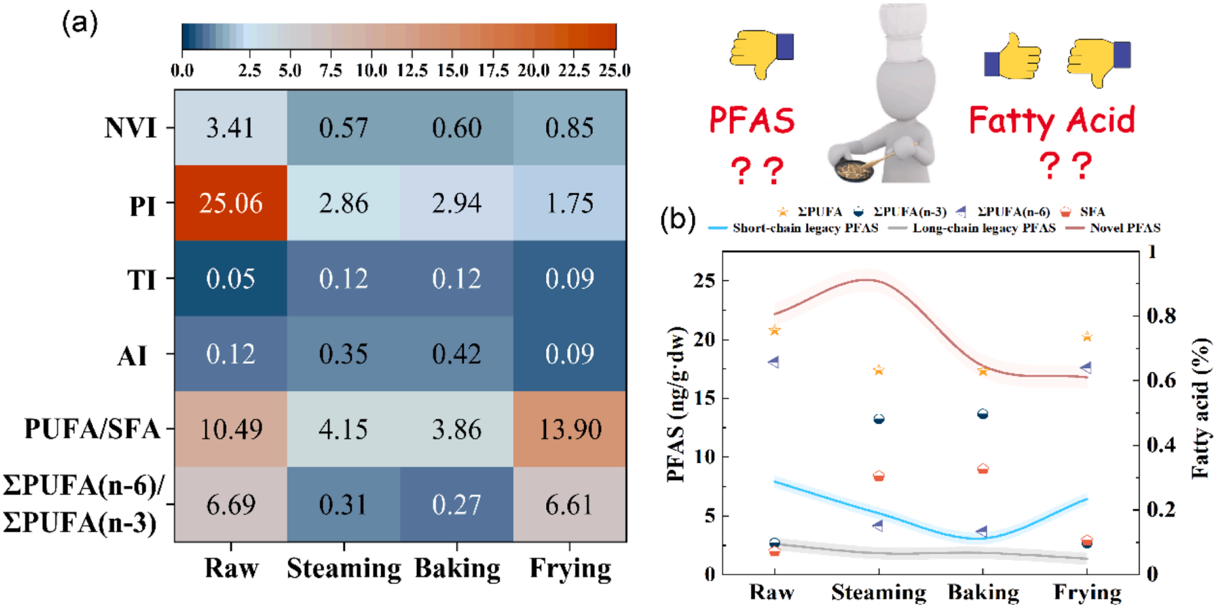

Fig. 6. The distribution of fatty acids and PFAS by different cooking processes. **Note: (a) The heat map of various indicators in cooked squids was shown; (b) The variations of PFAS and fatty acids were presented on the right-hand side. The dark line in the middle represented the average value, and the shaded part showed the five percent confidence interval.**

(DHA). According to the above description, squid is representative economical seafood. It has high nutritional value and a low cardiovascular disease risk index, so it appeared in this study, which was recommended as a kind of healthy food.

3.6. Potential risk of dietary PFAS from consumption

Dietary intake is the significant pathway of human exposure to PFAS. The short-chain and novel PFAS should be thought as the high levels found in the environment by increasing regulatory frameworks and subsequent adjustment in industrial use patterns (Abafe et al. 2021). EDI of novel PFAS vs. legacy PFAS by taking various tissues for residents was shown in Fig. 7. The mean EDI of PFBA, PFPeA, and PFOS were 2.63–45.33, 0.28–9.85, and 1.57–8.34 ng/kg bw/day, respectively (Table S10). In different tissues of squid, although risks of novel PFAS such as PFMOPrA, PFMOBA, and F-53B from squids were significantly lower than corresponding legacy PFAS (Mann-Whitney *U* test, $p < 0.05$). Based on the ratio between EDI and RfD data (Table S11) of PFAS, HRs of individual PFAS estimated health risks for human intake. In this study, the HRs were generally below 1 for PFAS in edible parts of squids. Nevertheless, the mean HR of PFPeA was 2.59 in the digestive system, which poses an adverse human health effect by taking the digestive system of squids. Therefore, it is recommended that squid should be eaten after cleaning the digestive system as much as possible to avoid human exposure to more PFAS.

Among tested PFAS by various cooked methods, EDI $_{\text{squids}}$ of PFBA were the highest ranging from 5.25 to 14.24 ng/kg bw/day in China (Table S12; Figure S9), and these values were lower than the RfD. Hence, the exposure of PFBA via different cooked squids for people currently did not induce a risk for human health which is similar to previous studies in the *Rana catesbia*na (Sun et al. 2021a). In the present study, the EDI values of PFOS (0–0.74 ng/kg bw/day) were significantly lower than those reported in raw and cooked grass carp (62.9–101 ng/kg bw/day) collected from Tangxun Lake, Wuhan, China (Hu et al. 2020).

The potential risks for human health via consuming squid were various in different countries based on basic information shown in Table S13. The EDI values of investigative PFAS ranged from 0 to 55.39 ng/kg bw/day (Table S14-17). Compared with different countries, Korea had the highest level of EDI via consuming squids through cooking processes, which was related to dietary habits and consumption of squids (Fig. 7). These values were all below RfD except for PFPeA in frying squids. PFPeA, as a short carbon-chain chain PFAS, had a high HR value in cooked squids, and the HR value was well above the safety threshold value "1″ which meant a high risk for human health. The EDI values of PFPeA at high levels also were found in marine shellfish, shrimp, vegetables, fruits, pork, and so on (Abafe et al. 2021; Gao et al. 2022). People can accumulate PFAS through different foods which could cause serious harm for humans. The short carbon-chain PFAS have been gradually producing and superseding the legacy PFAS, so human exposure to short carbon-chain PFAS will be expected to increase.

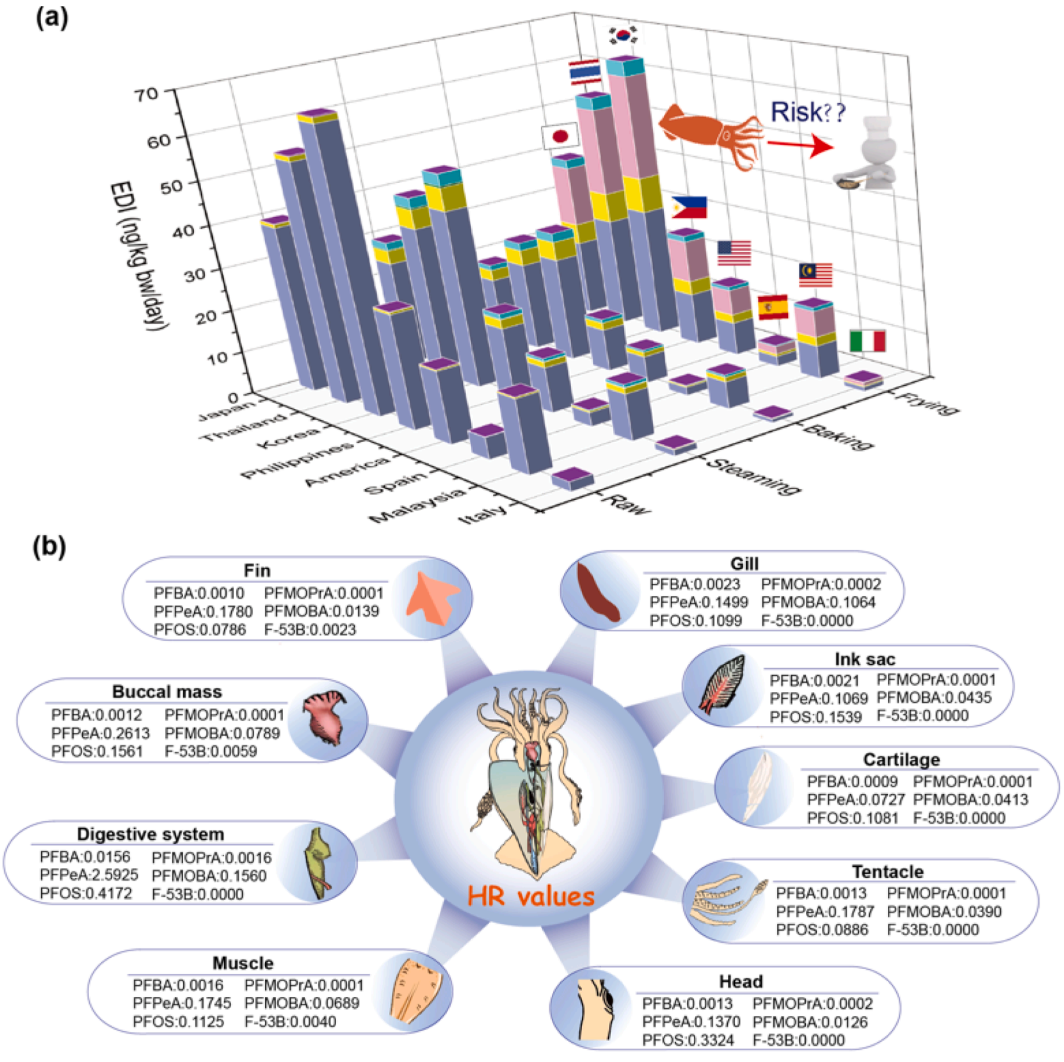

Fig. 7. The assessment of health risks in PFAS via squids.

Therefore, the potential risk of short-carbon chain PFAS should be kept in the spotlight.

4. Conclusions

Squid, as one popular blue food offering high protein and delicious cuisine, was analyzed for legacy and novel PFASs in raw and cooked tissues. Significant spatial variations in PFASs concentrations and compositions were elucidated. PFASs with a one hundred percent detectable ratio in all tissues, and the digestive system with the highest ability to accumulate PFASs in squids. PFBA and HFPO-TeA were the predominant PFAS detected in the biota.

There were significant changes in PFAS levels in squids due to differences in cooking methods, particularly when the inhabitants consumed this popular aquatic product of great concern for human health. The most effective cooking method to reduce PFAS was the baking process, followed by frying and steaming. Changes of PFAS in cooked oil and juice after cooking can explain the transfer mechanism between different media.

Based on the assessment of the nutritional index and disease risk, squids had high nutritional value that can be regarded as healthy seafood. The digestive system may cause high health risks to exposure PFPeA, so cleaning or peeling viscera before intake was recommended to avoid adverse effects. These conclusions can assist decision making on mitigation strategies of food hazards, provide basic information for risk assessments, and increase vigilance of food safety. In addition, some research needs to be further explored. The seasonal variation may be an underlying factor to affect the differences in PFASs concentrations in the tissues of squids, which will cause various health risks for humans. Hence, the health risks of consuming seafood in different seasons need to be further investigated in the future.

CRediT authorship contribution statement

Qiongping Sun: Investigation, Methodology, Formal analysis, Data curation, Writing – review & editing. **Tieyu Wang:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. **Xinyi Zhan:** Investigation, Formal analysis, Data curation, Writing – original draft. **Seongjin Hong:** Conceptualization, Writing – review & editing. **Lanfang Lin:** Investigation, Formal analysis, Writing – original draft. **Peixin Tan:** Investigation, Formal analysis, Writing – original draft. **Yonglong Xiong:** Investigation, Formal analysis, Writing – original draft. **Hancheng Zhao:** Investigation, Formal analysis, Writing – original draft. **Zhixin Zheng:** Investigation, Formal analysis, Writing – original draft. **Ran Bi:** Investigation, Formal analysis, Data curation. **Wenhua Liu:** Investigation, Formal analysis, Data curation. **Shuqi Wang:** Investigation, Formal analysis, Data curation. **Jong Seong Khim:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108024.

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Supplementary Material

Legacy and novel perfluoroalkyl substances in raw and cooked squids: Perspective from health risks and nutrient benefits

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Content:

Classification of PFAS

The long-chain PFAS covered perfluoroalkyl sulfonic acids ($C_nF_{2n+1}SO_3H$, $n \geq 6$, PFSAs) and perfluoroalkyl carboxylic acids ($C_nF_{2n+1}COOH$, $n \geq 7$, PFCAs) according to other research (Buck et al. 2011). The novel PFAS includes 2,2,3,3-tetrafluoro-3-(trifluoromethoxy) propionic acid (PFMOPrA), perfluoro (4-methoxybutanoic) acid (PFMOBA), sodium dodecafluoro-3H-4,8-dioxanonanoate (ADONA), chlorinated polyfluorinated ether sulfonate (F-53B) and perfluoro-(2,5,8-trimethyl-3,6,9-trioxadodecanoic) acid (HFPO-TeA). And legacy perfluoroalkyl substance (PFAS) covers perfluoro-butanesulfonate (PFBS), perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorobutyric acid (PFBA), perfluorodecane sulfonate (PFDS), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoate acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), and perfluorooctadecanoic acid (PFODA).

Chemical extraction and cleanup of PFAS

Biological samples

Squid samples were immediately dissected into the tentacle, buccal mass, head, ink sac, gill, cartilage, muscle, digestive system, and fin. All tools were soaked in a cleaning agent and washed, repeated three times with Milli-Q water and methanol before experiments to avoid experimental interference of cross-contamination. Experimental methods are shown in this research as follows (Diao et al., 2022; Sun et al., 2021). One gram of freeze-dried sample (raw squids or cooked squids) was spiked with 5 ng of internal standards (ISs), one milliliter of 0.5 M tetrabutylammonium hydrogensulfate (TBAHS) solution, and two milliliters of $NaHCO_3/Na_2CO_3$ buffer solution (pH 10). After sufficient mixing, the mixture was added with five milliliters of methyl-tert-butyl ether (MTBE) for extraction by shaking at 250 rpm for 15 min. Then the sample was centrifuged at 3000 rpm for 10 min to separate organic and aqueous layers. The liquid supernatant was transferred to a new polypropylene (PP) tube. The tube with the residue was extracted with five milliliters of MTBE repeated twice as above. All liquid supernatants were combined and concentrated under nitrogen gas.

To reduce interference, the sample was purified by solid-phase extraction (SPE) with ENVI-Carb and Oasis WAX. ENVI-Carb was preconditioned with three milliliters of methanol; the sample was transferred into the SPE cartridge. Then one milliliter of methanol was used to wash PP the tube and cartridge three times, respectively. All elution was collected in a PP tube and diluted to one hundred milliliters of Milli-Q water. The Oasis WAX was preconditioned with four milliliters of 0.1% NH4OH in methanol, followed by four milliliters of methanol and Milli-Q water. The sample and four milliliters of 25 mM ammonium acetate were loaded onto Oasis WAX in turn allowed to run dry. Four milliliters of methanol and 0.1% ammonia in methanol were used to wash Oasis WAX. Ultimately, eluents were concentrated under high-purity nitrogen and passed through a nylon filter (0.2 μm), then transferred into a 1.5 mL auto-sampler vial fitted with a PP cap for instrumental analysis.

Cooking oil samples

Experimental methods were used as reported in the previous study (Taylor et al., 2019). Zero point three milliliters of oils from the cooked sample and blank were spiked with 5 ng ISs, 0.6 mL of $MeOH/H_2O$ (1:1 V/V+0.5% NH_3H_2O), and 0.6 mL of dichloromethane. The mixture was shaken at 250 rpm for 5 min and centrifuged at 1200 rpm for 10 min. The liquid supernatant was transferred to a new 15 mL PP tube and loaded by SPE with Oasis WAX, following the same method as for the sample.

Juice

The procedure for PFAS analysis in juice samples was similar to the previous studies (Hu et al., 2020). One mL of the sample with five nanograms ISs and five mL of acetonitrile were added into a 15 mL PP tube for extraction by shaking at 250 rpm for 5 min and centrifuging at 3500 rpm for 10 min. The mixture was transferred to a PP tube, then 5 mL acetonitrile was added to the residue, and the extraction was repeated twice. The concentrated extract of juice was diluted to 100 mL of ultrapure water, then purified by a WAX cartridge for cleanup.

Analysis of fatty acids

Fatty acid concentrations were measured using a gas chromatography-mass spectrophotometer (GC/MS) according to previous methods (Zula et al., 2021). One half gram sample was added into a 10 mL glass tube with 6 mL of chloroform/methanol (2/1, v/v), and the mixture was stored at 4 °C in a refrigerator for two days. The sample was added to 2.7 mL ultrapure water by shaking at 250 rpm for 2 min and centrifuging at 3000 rpm for 10 min to separate organic and aqueous layers. All elution was collected in another 10 mL glass tube by Pasteur pipette, and the glass tube with the residue was extracted with 3 mL of chloroform repeated twice as above. All liquid supernatants were combined and concentrated under nitrogen gas.

Whereafter, 2.5 mL of 2% vitriol /methanol was added to the sample, and the mixture was heated at 85 °C for 3 h in a warm water bath. The mixture was cooled to room temperature after incubation and added with 1 mL of saturated sodium chloride and 2 mL of n-hexane. Finally, the sample was concentrated to 1 mL under high-purity nitrogen, then transferred into a 1.5 mL brown glass vial fitted with a PP cap for analysis.

Instrumental analysis

PFAS were analyzed using Thermo Ultimate 3000 Infinity HPLC System equipped with a Thermo TSQ ENDURA LC/MS System equipped with electrospray ionization (ESI) in negative ion mode and with multiple reaction monitoring (MRM) of the target analytes. Agilent ZORBAX Eclipse Plus C18, 2.1×100 mm, 3.5 μm (Agilent) connected with a guard column (Agilent) was used for chromatographic separation of legacy and novel PFAS. The categories of legacy and novel PFAS were listed in materials and reagents. Two mM ammonium acetate (A) and 100% acetonitrile (B) were used as the mobile phase. Five μL of the extracts was injected into the column at a flow rate of 0.3 mL/min.

Quality assurance and quality control

PFAS levels were used on dry weight bases for comprehending and unifying distribution patterns. “ND” represented the values below the linear dynamic range of the curve. The limit of detection (LOD) and limit of quantification (LOQ) was determined as the analyte peak that yielded a signal-to-noise ratio of 3:1 and 10:1, respectively. In the statistical analysis, all values lower than LOD were specified as LOD/√2, and in the case of concentrations lower than LOQ, the value of LOQ/√2 was used. The calibration curve consisted of a series of concentration gradients ensuring that the deviation between the determined value of each point and its theoretical value did not exceed 20%. Calibration curves in PFAS were 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 150, and 200 ng/mL, respectively. Calibration curves in fatty acids were 0.1, 0.5, 1, 5, 10, 15, and 20 mg/mL, respectively. The regression coefficients were > 0.999 in each calibration curve of individual PFAS.

Table S1. Annual consumption and output of squid in different countries

	Annual consumption (kiloton)	Annual output (kiloton)	The number of Chinese exports (kiloton)	Data source
Japan	400	82	88	https://comtrade.un.org/;https://www.statista.com/
Thailand	300	100	69.8	https://comtrade.un.org/; http://www.seafdec.org/
Korea	273	57	60.7	https://comtrade.un.org/; https://www.statista.com/
Philippines	200	54	29	https://comtrade.un.org/; http://www.seafdec.org/
America	-	49	29	https://comtrade.un.org/; https://www.fao.org
Spain	20	7.5	16.4	https://comtrade.un.org/; https://www.statista.com; https://www.fao.org
Malaysia	50	30	12.8	https://comtrade.un.org/; https://www.fao.org
Italy	11	4	5.8	https://comtrade.un.org/; https://www.statista.com; https://www.fao.org

Table S2. The information about size, number, maturation, and species of squids

	Number	ML (mm)	BW (g)	Maturation	Species	Data source
Small-size	10	174.3±24.2	129.7±26.7	Immature	*Uroteuthis edulis*	Yamaguchi et al. 2019
Large-size	10	288.2±67.3	338.0±119.5	Mature	*Uroteuthis edulis*	Yamaguchi et al. 2019
Small-size	72	159.5±13.3	109.8±15.8	Immature	*Uroteuthis edulis*	This study
Large-size	36	330.8±31.6	417.7±84.1	Mature	*Uroteuthis edulis*	This study

Table S3. Chemical formula, parent ion, quantitative ion and qualitative ion of PFAS

Analyte	Compound	Chemical formula	Parent Ion (m/z)	Quantitative ion (m/z)	Qualitative ion (m/z)
PFCAs					
PFBA	perfluoro-butanoate acid	$C_3F_7CO_2^-$	212.9	169.0	131.1
PFPeA	perfluoropentanoate acid	$C_4F_9CO_2^-$	262.9	218.9	141.0
PFHxA	perfluorohexanoate acid	$C_5F_{11}CO_2^-$	312.9	268.9	119.1
PFHpA	perfluoroheptanoate acid	$C_6F_{13}CO_2^-$	362.8	318.9	169.0
PFOA	perfluorooctanoate acid	$C_7F_{15}CO_2^-$	412.8	368.9	169.0
PFNA	perfluorononanoate acid	$C_8F_{17}CO_2^-$	462.8	418.9	218.9
PFDA	perfluorodecanoate acid	$C_9F_{19}CO_2^-$	512.8	468.9	218.7
PFUnDA	perfluoroundecanoate acid	$C_{10}F_{21}CO_2^-$	562.9	518.9	268.9
PFDoDA	perfluorododecanoate acid	$C_{11}F_{23}CO_2^-$	612.8	568.9	318.9
PFTrDA	perfluorotridecanoate acid	$C_{12}F_{25}CO_2^-$	662.8	618.9	318.9
PFTeDA	perfluorotetradecanoate acid	$C_{13}F_{27}CO_2^-$	712.8	668.9	368.9
PFHxDA	perfluorohexadecanoate acid	$C_{15}F_{31}CO_2^-$	812.8	768.8	368.9
PFODA	perfluorooctadecanoate acid	$C17F_{35}CO_2^-$	912.8	368.9	568.9
PFSAs					
PFBS	perfluorobutane sulfonate	$C_4F_9SO_3^-$	298.8	98.9	80.0
PFHxS	perfluorohexane sulfonate	$C_6F_{13}SO_3^-$	399.0	98.9	80.0

PFOS	perfluorooctane sulfonate	$C_8F_{17}SO_3^-$	498.9	99.0	80.0
PFDS	perfluorodecane sulfonate	$C_{10}F_{19}SO_3^-$	598.8	98.9	79.8
F-53B	chlorinated polyfluoroalkyl ether sulfonic acid	9CL-PF$_3$ONS	530.9	350.9	82.9
ADONA	3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid]	$C_7F_{12}O_4H^-$	376.0	251.1	85.2
PFMOPrA	perfluoro-4-oxapentanoic acid	$C_4F_7O_3H^-$	228.9	85.1	185.2
PFMOBA	perfluoro-5-oxahexanoic acid	$C_5F_9O_3H^-$	278.9	85.3	234.8
HFPO-TeA	perfluoro-(2,5,8-trimethyl-3,6,9-trioxadodecanoic) acid	$C_{13}F_{23}O_5H^-$	351.0	185.1	169.0
$^{13}C_4$ PFBA	$^{13}C_4$Perfluoro-butanoic acid		217.0	172.0	185.0
$^{13}C_4$PFHxA	$^{13}C_4$Perfluoro-hexanoic acid		314.9	255.2	296.9
$^{13}C_4$ PFOA	$^{13}C_4$Perfluoro-octanoic acid		416.8	371.9	168.9
$^{13}C_4$ PFNA	$^{13}C_4$Perfluoro-nonanoic acid		467.8	422.9	219.0
$^{13}C_4$ PFDA	$^{13}C_4$Perfluoro-decanoic acid		514.8	469.9	268.9
$^{13}C_4$PFUDA	$^{13}C_4$Perfluoro-undecanoicacid		564.8	519.9	268.9
$^{13}C_2$PFDoA	$^{13}C_4$Perfluoro-dodecanoicacid		614.8	569.9	270.1
$^{18}O_2$ PFHxS	$^{18}O_2$Perfluorohexanesulfonate		402.8	84.1	103.0
$^{13}C_4$ PFOS	$^{13}C_4$Perfluoro-octanesulfonate		502.8	104.2	80.0

Table S4. HPLC-ESI-MS instrument conditions

HPLC conditions		
analytical column	Aglient ZORBAX Eclipse Plus C18, 2.1×100 mm, 3.5 µm	
guard column	Agilent 1290 Infinity In-line filter with 0.3 µm SS frit	
injection volume	5 µL	
column temperature	40 °C	
mobile phase	A = 2 mM ammonium acetate	
	B = 100% Acetonitrile	
run time	16 min + 4 min post time	
flow rate	0.3 mL/min	
gradient	Time (min)	Mobile phase
	0	20% B
	14	90% B
	16	90% B
MS conditions		
acquisition parameters	ESI mode, negative ionization; MRM	
source gas temperature	350 °C	
source gas flow rate	9 L/min	
nebulizer pressure	40 psi	
capiliary	3500 V positive; 4000 V negative	
delta EMV(-)	200-400 V	

Table S5. GC-MS instrument conditions

GC conditions		
analytical column	Shimadzu GCMS-QP2010SE, 30 m× 0.25 mm× 0.25μm	
injection volume	1.0μL	
column temperature	250 °C	
instrument pressure	112.5 kPa	
split ratio	1.0	
gradient	retention time (min)	temperature (°C)
	5	100
	1	180
	2	220
	5	230
	3	250
MS conditions		
acquisition parameters	EI mode; Scan	
source gas temperature	325°C	
source gas flow rate	9 L/min	
nebulizer pressure	40 psi	
mass sweep range	35-600 m/z	

Table S6. Quality assurance and quality control (QA/QC) for PFAS in this study

	LOD [a] (ng/g.dw)	**LOQ [b] (ng/g.dw)**	**Recovery (%)**		
			Biota	**Juice**	**Oil**
PFBA	1.07E-02	3.57E-02	0.94	0.65	0.71
PFMOPrA	1.15E-03	3.85E-03	0.61	0.62	0.59
PFPeA	1.25E-03	4.17E-03	0.82	0.57	0.63
PFMOBA	1.07E-03	3.57E-03	0.75	1.09	0.95
PFHxA	2.68E-03	8.93E-03	0.76	0.73	0.82
PFHpA	2.42E-03	8.06E-03	1.02	0.88	0.69
L-PFBS	9.38E-03	3.13E-02	1.21	0.89	0.70
ADONA	4.84E-03	1.61E-02	1.00	1.01	0.74
PFOA	1.36E-02	4.55E-02	1.19	0.75	0.70
L-PFHxS	2.34E-03	7.81E-03	1.22	0.85	0.71
PFNA	9.38E-03	3.13E-02	1.17	0.86	0.72
PFDA	1.36E-02	4.55E-02	1.23	0.81	0.68
PFOS	6.52E-03	2.17E-02	1.31	0.72	0.76

F-53B	1.49E-03	4.95E-03	1.24	0.86	0.68
PFUdA	1.15E-03	3.82E-03	1.21	0.91	0.69
L-PFDS	1.00E-02	3.33E-02	1.00	0.87	0.76
PFDoA	2.42E-03	8.06E-03	1.28	0.93	0.71
PFTrDA	1.00E-03	3.33E-03	0.85	0.72	0.69
PFTeDA	2.34E-03	7.81E-03	1.18	0.69	0.64
PFHxDA	3.13E-03	1.04E-02	1.25	0.87	0.68
PFODA	3.66E-03	1.22E-02	1.31	0.83	0.62
HFPO-TeA	7.43E-05	1.24E-04	1.08	1.01	0.73

Note: a, The LOD was defined as the amount of chemicals which could be detected in a given amount of samples after the entire method was performed (SNR=3:1); b, LOQ was defined as the minimum concentration of a substance that can be quantitatively measured in the specific product with an acceptable level of accuracy and precision (SNR=10:1).

Table S7. PFAS concentrations in juice and oil (ng/L)

	Juice	**Oil**	**Blank juice**	**Blank oil**
PFBA	0.00	1.37	0.00	0.90
PFMOPrA	0.00	0.14	0.11	0.00
PFPeA	0.00	0.04	0.00	0.08
PFMOBA	0.02	0.00	0.00	0.00
PFHxA	0.06	0.04	0.06	0.01
PFHpA	0.01	0.02	0.02	0.02
PFBS	0.00	0.05	0.00	0.07
ADONA	0.00	0.00	0.01	0.00
PFOA	0.02	0.03	0.00	0.05
PFHxS	0.00	0.00	0.04	0.00
PFNA	0.01	0.03	0.00	0.02
PFDA	0.01	0.01	0.00	0.00
PFOS	0.00	0.03	0.05	0.00
F-53B	0.00	0.02	0.00	0.00
PFUdA	0.01	0.00	0.03	0.02
PFDS	0.00	0.00	0.00	0.00
PFDoA	0.00	0.01	0.03	0.00
PFTrDA	0.04	0.01	0.02	0.02
PFTeDA	0.02	0.04	0.05	0.01
PFHxDA	0.03	0.03	0.03	0.04
PFODA	0.00	0.00	0.00	0.02
HFPO-TeA	0.81	9.51	0.62	9.18
ΣPFAS	1.04	11.37	1.06	10.45
Short-chain legacy PFAS	0.07	1.52	0.11	1.07
Long-chain legacy PFAS	0.14	0.18	0.21	0.19
Novel PFAS	0.83	9.67	0.74	9.19

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Table S8. The index of fatty acids in squids of different sites

	ΣPUFA	ΣPUFA (n-3)	ΣPUFA (n-6)	SFA	ΣPUFA+ ΣMUFA	ΣMUFA	ΣPUFA (n-6)/ (n-3)	PUFA/ SFA	NVI	AI	TI	PI
ZJ1	75%	9%	66%	7%	93%	18%	7.07	11.41	0.38	0.09	0.03	28.62
ZJ2	78%	11%	67%	6%	94%	16%	5.92	12.66	2.84	0.11	0.05	20.29
ST1	76%	10%	66%	7%	93%	17%	6.69	10.49	3.41	0.12	0.05	25.06
ST2	63%	9%	54%	5%	95%	32%	5.67	13.34	4.36	0.01	0.02	21.09
QZ1	75%	10%	65%	6%	94%	19%	6.37	12.55	3.41	0.01	0.02	16.83
QZ2	68%	15%	52%	11%	89%	21%	3.46	6.05	2.65	0.19	0.07	16.98
ZS1	76%	12%	64%	7%	93%	16%	5.37	10.39	7.27	0.03	0.02	47.45
ZS2	61%	14%	46%	13%	87%	27%	3.24	4.79	4.52	0.17	0.07	22.61
LYG1	68%	9%	59%	23%	77%	10%	6.85	3.00	0.06	0.23	0.27	0.54
LYG2	78%	10%	69%	7%	93%	14%	7.23	10.63	5.00	0.04	0.02	43.42
QD1	78%	12%	67%	7%	93%	15%	5.80	10.82	6.25	0.03	0.02	54.30
QD2	60%	39%	21%	37%	63%	3%	0.55	3.24	0.43	0.23	0.10	3.27
Mean	71%	13%	58%	11%	89%	17%	5.35	9.11	3.38	0.11	0.06	25.04

Note: ZJ, ST, QZ, ZS, LYG, and QD represent Zhanjiang City, Shantou City, Quanzhou City, Zhoushan City, Lianyungang City, and Qingdao City, respectively.1 and 2 represent large and small squids, respectively.

Table S9. The index of fatty acids in squids by different cooking methods

	Raw	**Steaming**	**Baking**	**Frying**
ΣPUFA	76%	63%	63%	74%
ΣPUFA(n-3)	10%	48%	50%	10%
ΣPUFA(n-6)	66%	15%	13%	64%
ΣSFA	7%	30%	33%	11%
ΣPUFA＋ΣMUFA	93%	70%	67%	89%
ΣMUFA	17%	6%	4%	16%
ΣPUFA(n-6)/ΣPUFA(n-3)	6.69	0.31	0.27	6.61
PUFA/SFA	10.49	4.15	3.86	13.90
AI	0.12	0.35	0.42	0.09
TI	0.05	0.12	0.12	0.09
PI	25.06	2.86	2.94	1.75
NVI	3.41	0.57	0.60	0.85

Table S10. Estimated daily intake (EDI) of PFAS in various tissues

		PFBA	PFMO -PrA	PFPeA	PFMO -BA	PFOS	F-53B
Muscle	Min	0.00	0.00	0.00	0.00	0.00	0.00
	Max	10.83	0.48	3.31	2.12	10.03	0.31
	Mean	4.50	0.20	0.66	0.26	2.25	0.08
Fin	Min	1.07	0.00	0.06	0.00	0.00	0.00
	Max	7.25	0.46	1.81	0.29	7.97	0.23
	Mean	2.91	0.23	0.68	0.05	1.57	0.05
Buccal mass	Min	0.57	0.00	0.00	0.00	0.00	0.00
	Max	7.29	0.75	4.09	0.78	23.56	0.66
	Mean	3.34	0.21	0.90	0.27	2.84	0.11
Head	Min	1.11	0.00	0.03	0.00	0.52	0.00
	Max	11.75	2.98	1.82	0.17	63.34	0.00
	Mean	3.87	0.67	0.52	0.05	6.65	0.00
Tentacle	Min	1.07	0.00	0.19	0.00	0.00	0.00
	Max	7.91	0.56	3.26	0.65	9.29	0.00
	Mean	3.63	0.21	0.68	0.15	1.77	0.00
Ink sac	Min	1.01	0.00	0.09	0.00	0.00	0.00
	Max	43.24	0.64	1.80	0.80	13.39	0.00
	Mean	6.01	0.22	0.41	0.17	3.08	0.00
Digestive system	Min	0.69	0.00	0.08	0.01	0.50	0.00
	Max	238.66	43.06	36.73	3.86	37.34	0.00
	Mean	45.33	4.76	9.85	0.59	8.34	0.00
Cartilage	Min	1.45	0.00	0.04	0.00	0.00	0.00
	Max	5.33	0.40	0.62	0.59	17.94	0.00
	Mean	2.63	0.18	0.28	0.16	2.16	0.00
Gill	Min	0.67	0.00	0.13	0.00	0.00	0.00
	Max	24.04	1.95	2.39	1.43	15.03	0.00
	Mean	6.61	0.71	0.57	0.40	2.20	0.00

Table S11. Established reference doses (RfD) of individual PFAS

Chemical compound	RfD (ng/kg·bw/day）	Data sources
PFBA	2900	https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfba2summ.pdf.
PFPeA	3.8	https://www.tceq.texas.gov/assets/public/implementation/tox/evaluations/pfcs.
PFOS	20	https://www.epa.gov/sites/default/files/2016-05/documents/pfos_health_advisory_final-plain.pdf

Note: PFMOPrA, PFMOBA, and F-53B have a similar chain and chemical properties to PFBA, PFPeA and PFOS, respectively. So the RfDs of PFMOPrA, PFMOBA, and F-53B referenced the values PFBA, PFPeA and PFOS, respectively.

Table S12. Estimated daily intake (EDI) via various cooking squids in China

	PFBA	PFMOPrA	PFOS	F-53B
Raw	14.24	0.29	0.00	0.06
Steaming	8.97	1.15	0.60	0.01
Baking	5.25	0.94	0.45	0.00
Frying	6.49	1.75	0.74	0.01

Table S13. The average weight (kg) in various countries

	Average weight (kg)	Data sources
Japan	61.4	The National Health and Nutrition Survey in Japan[a]
Thailand	58.05	(Jitnarin et al. 2014)
Korea	60.72	(Jang and Koh 2020)
Philippines	51.2	(Kuzawa et al. 2012)
America	76.7	(Mitchell et al. 2014)
Spain	62.4	(Bibiloni et al. 2017)
Malaysia	68.3	(Yang et al. 2016)
Italy	65.05	(Krul et al. 2011)
China	57	(Diao et al.2022)

a: Ministry of Health, Labour and Welfare, Japan. The National Health and Nutrition Survey in Japan, 2016. 2016. https://www.mhlw.go.jp/bunya/kenkou/eiyou/h28-houkoku.html

Table S14. Estimated daily intake (EDI) via raw squids in different countries

	PFBA	PFMOPrA	PFPeA	PFMOBA	PFOS	F-53B
Japan	38.76	0.80	0.00	0.00	0.00	0.15
Thailand	55.39	1.14	0.00	0.00	0.00	0.22
Korea	65.14	1.34	0.00	0.00	0.00	0.26
Philippines	26.38	0.54	0.00	0.00	0.00	0.11
America	16.62	0.34	0.00	0.00	0.00	0.07
Spain	5.00	0.10	0.00	0.00	0.00	0.02
Malaysia	16.75	0.34	0.00	0.00	0.00	0.07
Italy	2.15	0.04	0.00	0.00	0.00	0.01

Table S15. Estimated daily intake (EDI) via steaming squids in different countries

	PFBA	PFMOPrA	PFPeA	PFMOBA	PFOS	F-53B
Japan	24.43	3.14	0.00	0.24	1.62	0.04
Thailand	34.91	4.49	0.00	0.34	2.32	0.05
Korea	41.06	5.28	0.00	0.40	2.73	0.06
Philippines	16.63	2.14	0.00	0.16	1.11	0.03
America	10.47	1.35	0.00	0.10	0.70	0.02
Spain	3.15	0.41	0.00	0.03	0.21	0.00
Malaysia	10.55	1.36	0.00	0.10	0.70	0.02
Italy	1.35	0.17	0.00	0.01	0.09	0.00

Table S16. Estimated daily intake (EDI) via baking squids in different countries

	PFBA	PFMOPrA	PFPeA	PFMOBA	PFOS	F-53B
Japan	14.29	2.56	0.00	0.00	1.22	0.00
Thailand	20.42	3.66	0.00	0.00	1.74	0.00
Korea	24.02	4.31	0.00	0.00	2.05	0.00
Philippines	9.73	1.74	0.00	0.00	0.83	0.00
America	6.13	1.10	0.00	0.00	0.52	0.00
Spain	1.84	0.33	0.00	0.00	0.16	0.00
Malaysia	6.17	1.11	0.00	0.00	0.53	0.00
Italy	0.79	0.14	0.00	0.00	0.07	0.00

Table S17. Estimated daily intake (EDI) via frying squids in different countries

	PFBA	PFMOPrA	PFPeA	PFMOBA	PFOS	F-53B
Japan	17.68	4.77	13.96	0.00	2.01	0.02
Thailand	25.27	6.82	19.95	0.00	2.88	0.02
Korea	29.71	8.02	23.46	0.00	3.38	0.03
Philippines	12.03	3.25	9.50	0.00	1.37	0.01
America	7.58	2.05	5.98	0.00	0.86	0.01
Spain	2.28	0.62	1.80	0.00	0.26	0.00
Malaysia	7.64	2.06	6.03	0.00	0.87	0.01
Italy	0.98	0.26	0.77	0.00	0.11	0.00

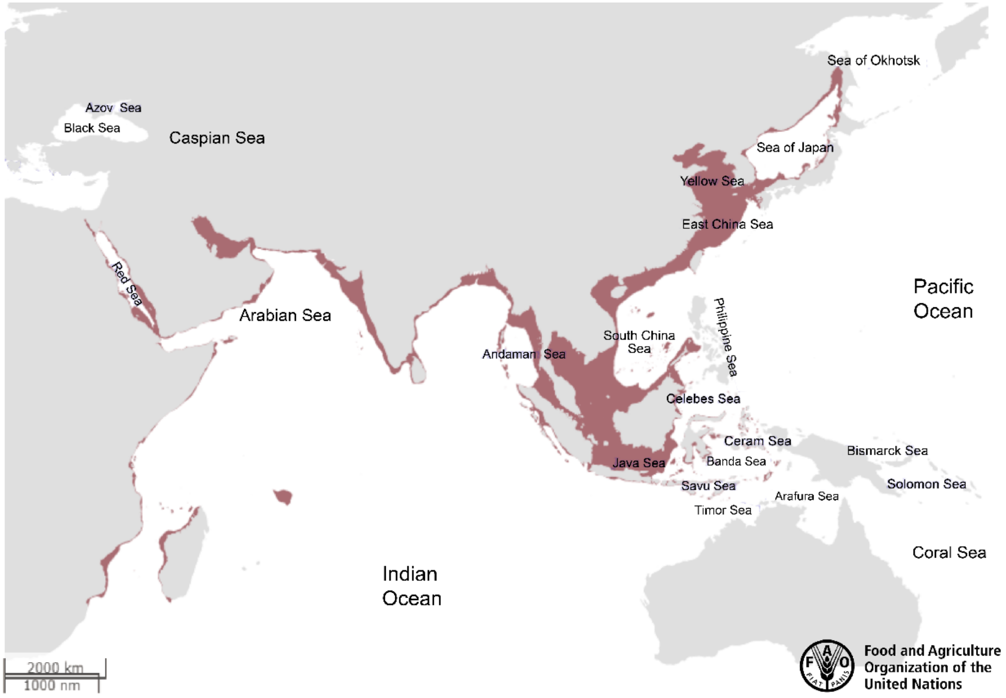

Figure S1. Global distribution map of squids (*Uroteuthis edulis)*

Note: Brown areas represent where squid are distributed. The data were obtained from https://www.fao.org/figis/geoserver/factsheets/species.html#speciesSelectorPanel

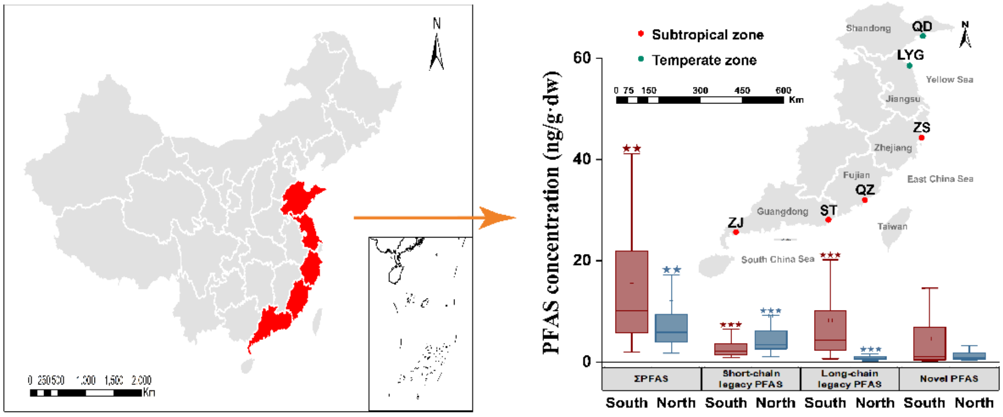

Figure S2. The sampling sites of biotas include large and small squids

Note: ★★★ means p is less than 0.001. ★★ means p is less than 0.01. The medians were shown in middle position

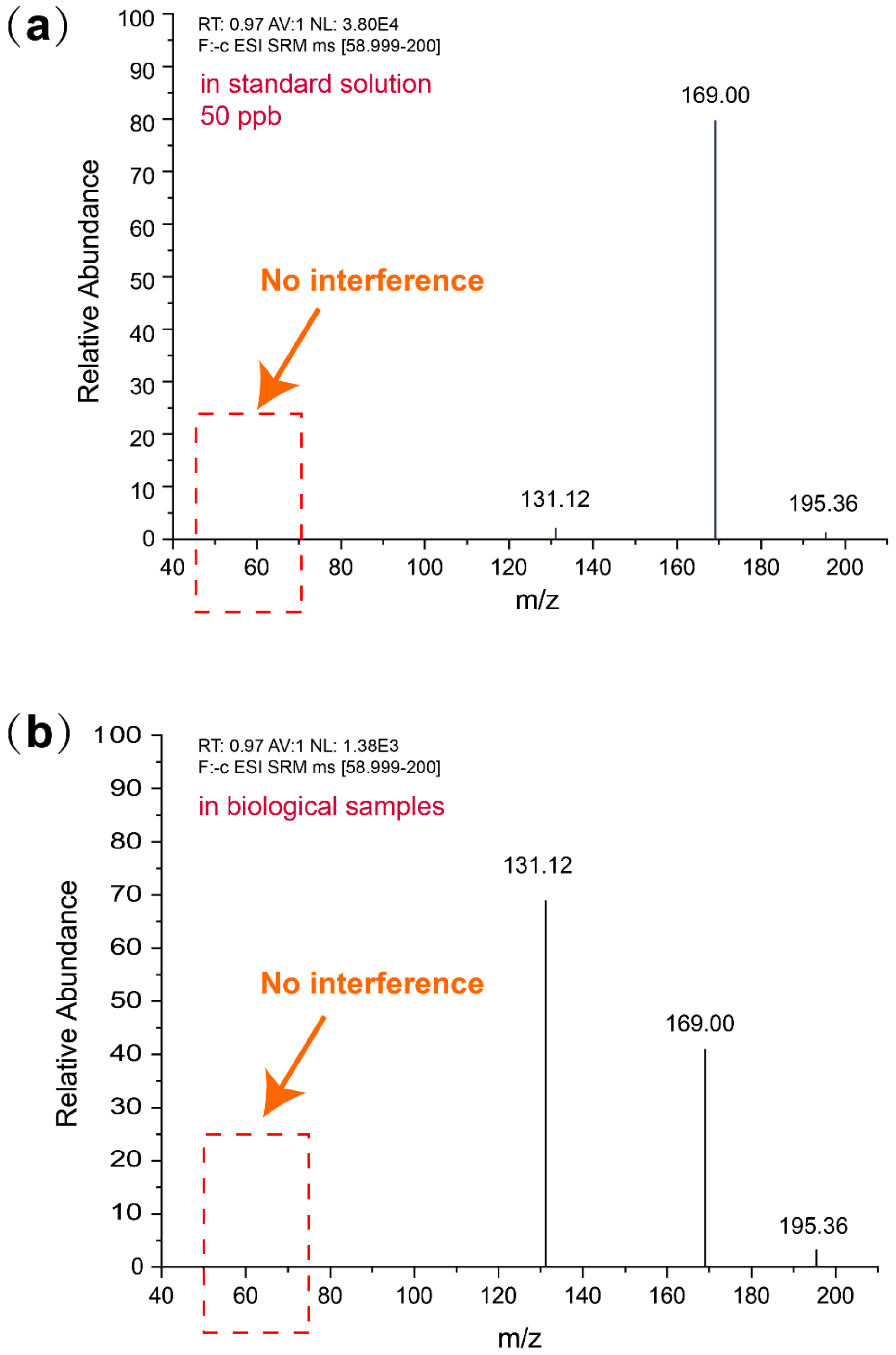

Figure S3. The information on mass spectrum in standard solution and biological samples

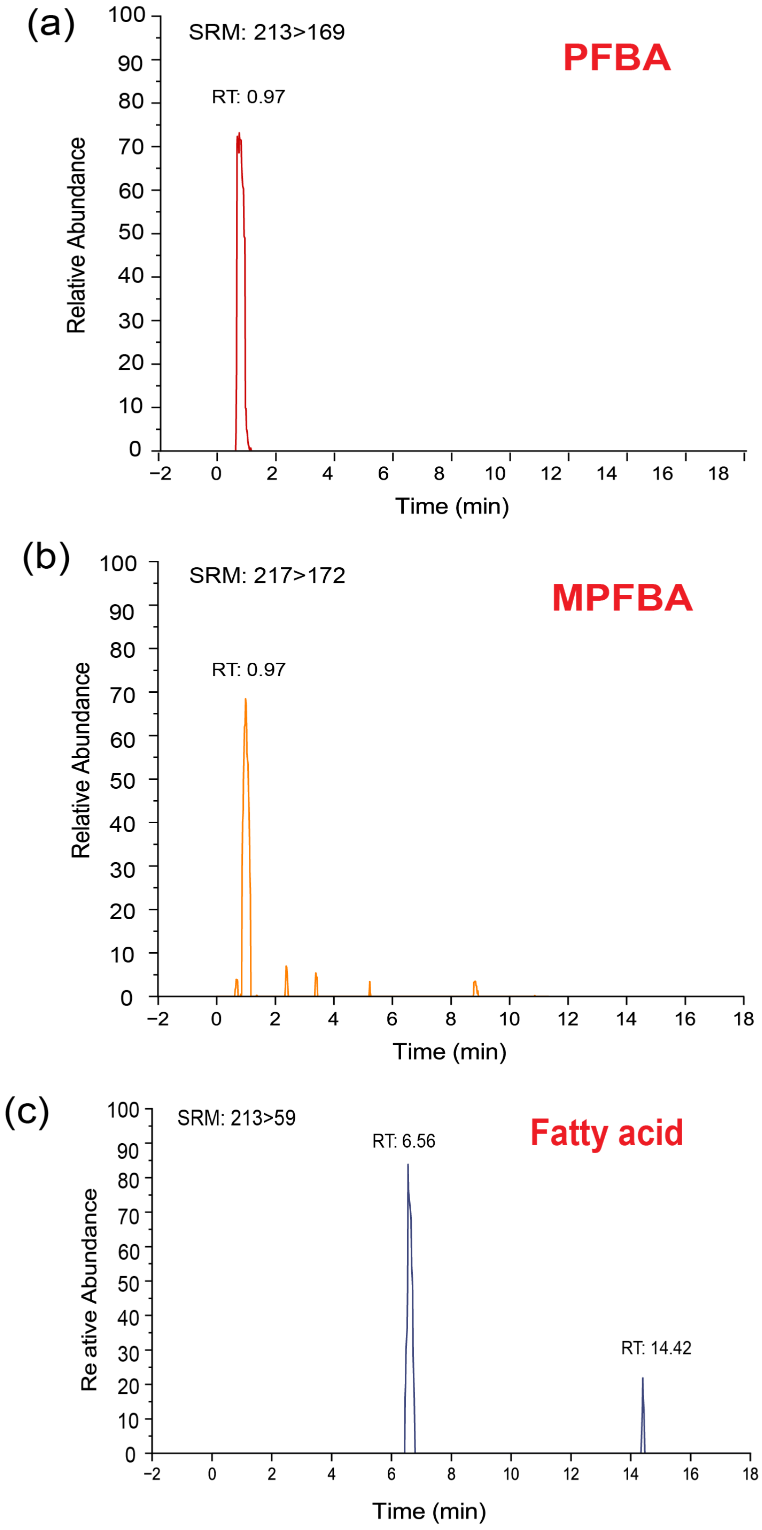

Figure S4. Confirmation analysis of PFBA, $^{13}C_4$PFBA, and fatty acid

Note: (a) is PFBA; (b) is $^{13}C_4$PFBA; (c) is fatty acid.

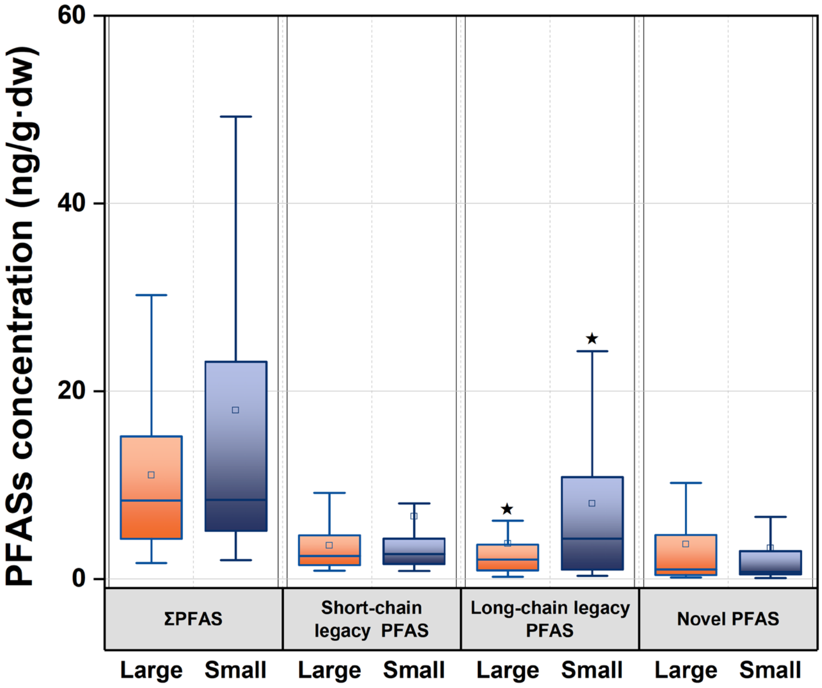

Figure S5. PFAS concentration in different sizes including large and small squids

Note: ★ means p is less than 0.05. The medians were shown in the middle position.

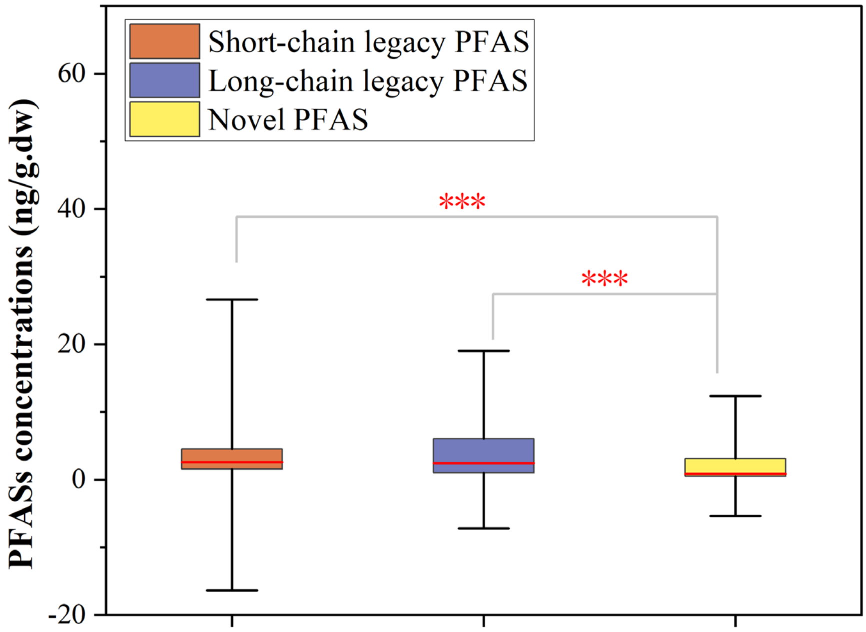

Figure S6. The comparisons of individual PFAS concentration (n=108)

Note: ★★★ means p is less than 0.001. The box ranged from 25% to 75%. The medians were shown in the middle position.

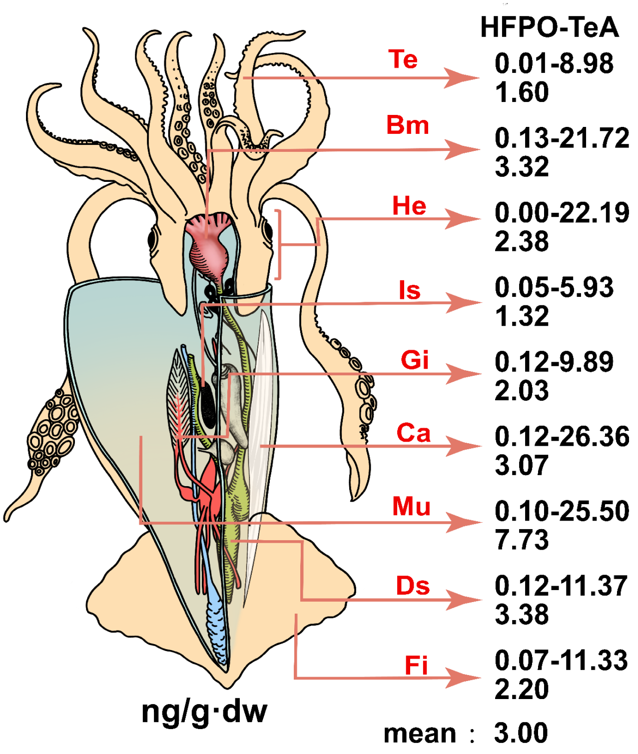

Figure S7. HFPO-TeA concentration in different tissues

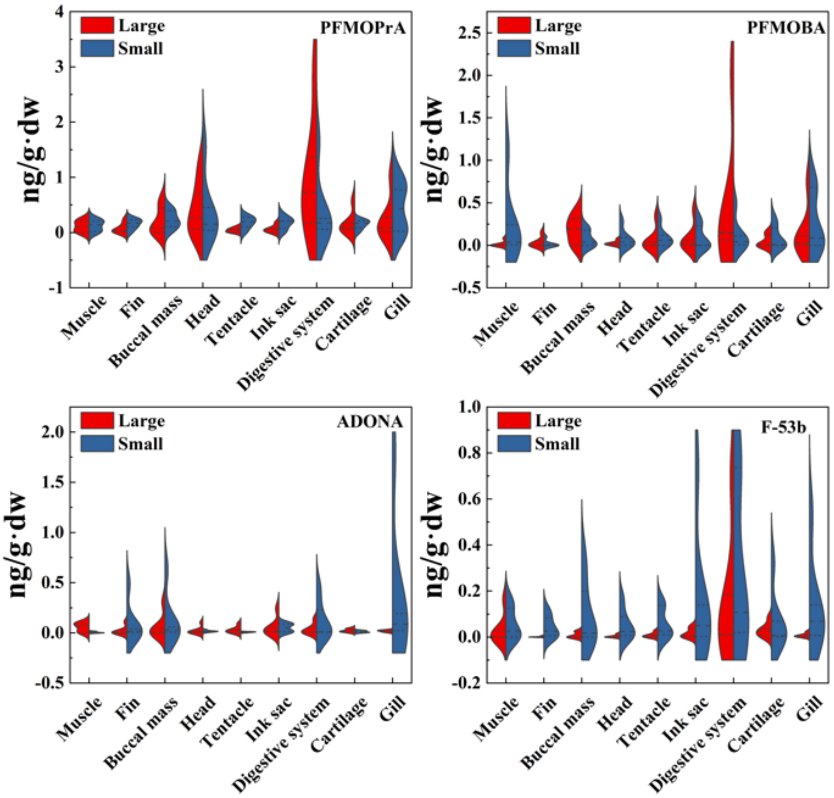

Figure S8. The distribution pattern of novel PFAS in small and large squids

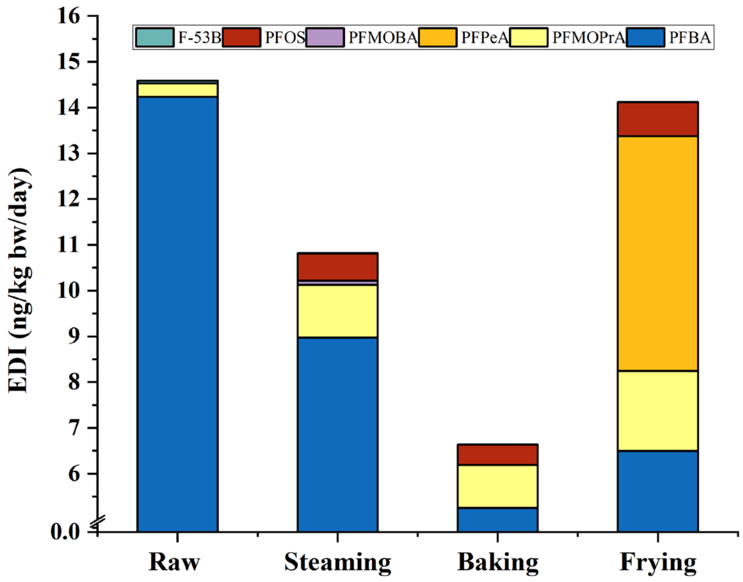

Figure S9. Estimated daily intake (EDI) of PFAS via cooking squids in China

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