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Analytical Methods

Halogenated polycyclic aromatic hydrocarbons in edible aquatic species of two Asian countries: Congener profiles, biomagnification, and human risk assessment

Anura Upasanta-Kumara Wickrama-Arachchige [a], Keerthi S. Guruge [b,c,*], Yuriko Inagaki [d], Hinako Tani [e], Tilak Siri Dharmaratne [a,f], Yasuaki Niizuma [e], Takeshi Ohura [d,e,*]

[a] Faculty of Fisheries and Ocean Sciences, Ocean University of Sri Lanka, Mahawela Road, Tangalle, HB 82200, Sri Lanka
[b] Pathology and Pathophysiology Research Division, National Institute of Animal Health, National Agriculture and Food Research Organization, 3-1-5 Kannondai, Tsukuba 305-0856, Japan
[c] National Institute of Fundamental Studies, Hanthana Road, Kandy, Sri Lanka
[d] Faculty of Agriculture, Meijo University, 1-501 Shiogamaguchi, Nagoya 468-8502, Japan
[e] Graduate School of Agriculture, Meijo University, 1-501 Shiogamaguchi, Nagoya 468-8502, Japan
[f] Gem and Jewellery Research and Training Institute, 73 /5/A, Kaduwela, Sri Lanka

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ABSTRACT

Seventy-five contaminants including chlorinated/brominated/parent polycyclic aromatic hydrocarbons (Cl/Br/PAHs) were investigated in 29 edible aquatic species from the Indian Ocean near Sri Lanka and 10 species from the Pacific Ocean near Japan. Concentrations of total ClPAHs and BrPAHs in the samples were 2.6–57 and 0.30–9.5 ng/g-dry weight from the Indian Ocean, and 0.35–18 and 0.03–3.3 ng/g-dry weight from the Pacific Ocean, respectively. Comparing the profiles of Cl/BrPAHs among the samples, congeners of chlorinated and brominated pyrene were predominant components and enhanced the potential for biomagnification in the sample from the off-shore pelagic environment in the Indian Ocean. The incremental lifetime cancer risks estimated by intake of the targets in consuming aquatic organisms showed that approximately one-third of studied organisms exceeded the acceptable risk level for Sri Lankans.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitously abundant in the universe as a naturally occurring organic compound, together with a considerable proportion that has originated due to anthropogenic activity (Boström et al., 2002). Some halogenated polycyclic aromatic hydrocarbons (HPAHs), such as chlorinated PAHs (ClPAHs) and brominated PAHs (BrPAHs), may be present in the environment at approximately 10–100 times lower than their corresponding PAHs, although their mechanisms of production are thought to be similar (Ohura et al., 2008, 2019). Several ClPAH and BrPAH congeners show toxic effects similar to those of dioxins (Ohura et al., 2009). Recently, we reported high levels of ClPAHs and BrPAHs in tuna fish, detected using a novel high-resolution Gas Chromatography (GC) Orbitrap Mass Spectrometry (MS)-based method (Wickrama-Arachchige et al., 2020). The oceanic and estuarine realms have been exposed to a vast array of anthropogenic and natural pollutants, being the ultimate repository for various organic substances, threatening their inhabitant fauna. Seafood is a source of nutrition for many people (Ferrante et al., 2018), and the consumption of contaminated species is one way for toxic chemicals to enter the human body, causing negative health effects (Ferrante et al., 2018). Therefore, the detection of high levels of HPAH congeners in tuna has prompted further investigations into their accumulation potential in other marine biotas, as an important new class of organic micropollutants.

Several perspectives regarding the bioaccumulation of PAHs in biota were suggested while the accumulation patterns of HPAHs in biota were not well known. Although the chlorinated organic compounds such as PCBs and dioxins exhibit an elevated risk with increasing trophic levels as a result of bioaccumulation, PAHs tend to decrease because of

* Corresponding authors at: Pathology and Pathophysiology Research Division, National Institute of Animal Health, National Agriculture and Food Research Organization, Japan (K.S. Guruge). Graduate School of Agriculture, Meijo University, Japan (T. Ohura).
E-mail addresses: guruge@affrc.go.jp (K.S. Guruge), ohura@meijo-u.ac.jp (T. Ohura).

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biodilution, that they showed a low assimilation efficiency and high metabolic transformation efficiency at higher trophic levels (Wan et al., 2007). The biodilution of PAHs has previously been observed in aquatic species (Qin et al., 2020). In contrast, some studies revealed a biomagnification potential of PAHs (Wang et al., 2012). Therefore, further studies evaluating the biomagnification potentials of PAHs are essential to address these discrepancies. To our knowledge, there is no evidence of the bioaccumulation pattern of HPAHs in aquatic species, which is a new class of environmental contaminants. Therefore, primarily, in this study, we hypothesized that there could be a significant positive relationship between the concentrations of HPAHs and PAHs in edible aquatic organisms. Secondly, we hypothesized that HPAHs may also show biomagnification similar to PAHs in the tissues of aquatic organisms. In the current study, a trophic magnification factor (TMF) which is a reliable tool to evaluate bioaccumulation of chemicals in environmental samples was used (Borgå et al., 2012). Moreover, a stable isotope analysis (SIA) was carried out for the Indian Ocean samples to determine the stable nitrogen ($\delta^{15}N$) values to determine the trophic level-specific accumulation properties of the target compounds.

Fish have long been used as a universal bioindicator for monitoring aquatic environmental pollution (Sarkar, Ray, Shrivastava, & Sarker, 2006) besides their usage as a source of protein and other necessary nutrients for the maintenance of a healthy human body. As pollutants, PAHs have been widely investigated in aquatic biota around the world, so their intake risks have been estimated (Martí-Cid et al., 2007). To date, HPAHs have been frequently found in fish (Masuda et al., 2019; Wickrama-Arachchige et al., 2020). HPAHs levels and their congeneric accumulation in aquatic biota could vary according to the sampling region, type of species, and trophic level (Wickrama-Arachchige et al., 2020). Therefore, the aims of this study were (i) investigate 51 HPAH congeners (30 ClPAH congeners and 21 BrPAH congeners) and 24 PAH congeners in edible aquatic species collected in the Indian Ocean ecosystems near Sri Lanka (29 species) and the Pacific Ocean off of Japan (10 species); (ii) examine accumulation potentials of congeners considering the number of rings to trace the origin of compounds; (iii) examine biomagnification potentials of both HPAHs and PAHs in aquatic edible species collected from the Indian Ocean by determining their trophic levels, and (iv) evaluate any toxic effects due to consumption of aquatic biota, where we calculated toxic equivalency quotients (TEQs) for target compounds in the specimens and estimated the incremental lifetime cancer risk (ILCR) for people living in Sri Lanka. This study was primarily aimed at revealing that HPAHs have the potential for biomagnification. To the best of our knowledge, this is the first study to investigate the occurrence of HPAHs in various aquatic species collected from two regions and describe the biomagnification processes of HPAHs based on trophic levels.

2. Materials and methods

2.1. Chemicals

The target compounds of this study were ClPAHs, BrPAHs, and PAHs, with the number of congeners being 30, 21, and 24, respectively. The details targeted in individual compounds are listed in Table S1. A standard mixture of 24 PAHs was purchased from LGC Labor GmbH (Augsburg, Germany), in which 16 of the US Environmental Protection Agency (US-EPA) priority PAHs are included. Almost all HPAHs targeted were originally synthesized and purified by Ohura et al; the details are described elsewhere (Kamiya et al., 2015; Ohura et al., 2005, 2009). The deuterated internal standards were phenanthrene-d_{10} and perylene-d_{12}, while the recovery standard for HPAHs and PAHs was fluoranthene-d_{10}. Labeled standards were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Analytical grade chemicals were used to carry out all extractions and cleanup activities; they were purchased from Wako Pure Chemical (Osaka, Japan) and Kanto Chemical (Tokyo, Japan).

2.2. Sample collection and procedures

Fresh samples of 30 aquatic organisms representing off-shore pelagic, coastal, and estuarine ecosystems were collected from a local market in Sri Lanka (Indian Ocean samples). The samples included finfishes (25 species of bony fishes, 1 species of ray and, 1 species of shark), and shellfishes (1 species of squid, and 1 species of shrimp). They were assumed to be represented different trophic levels due to their different feeding habits (Table S2). Two different geographic locations that the fish are living were considered when collecting Sri Lankan samples (i.e. off-shore pelagic which is away from the mainland and coastal and estuarine which is near to the mainland). The Sri Lankan samples represented 18 different species (19 specimens; only one tuna species consisted of each of a small and a large individual), from the off-shore pelagic environment near Sri Lanka (OPSL), and 11 from the coastal and estuarine environment near Sri Lanka (CESL). Besides, ten marine species including finfishes (8 species of bony fishes), and shellfishes (1 species of squid, and 1 species of scallop) were collected from a fish market from Japan (MJP) (Pacific Ocean samples). One specimen from the large species was randomly chosen for the analysis while several same-size specimens were pooled to prepare homogenized samples for small-sized species, which aimed to cover a wide range of biota, to obtain deviated congener-specific data from many species (Table S2). The yellowfin tuna represented the most popular fish of both countries, therefore 1 specimen for Japan and 2 specimens of different sizes were chosen for the Sri Lankan samples. Previously, a similar study on the trophic transfer of persistent toxic substances had been performed in a coastal food web in South Korea (An et al., 2020), and pooled aquatic biota samples were used to analyze PAHs (Qin et al., 2020). The consumption of aquatic species in these two countries is common and the collected species were among the most popular edible aquatic species available in both countries. This sampling strategy, therefore, enables the identification of the congeneric specificity in the edible aquatic species consumed by two Asian countries and the level of HPAHs and PAHs pollution of two different ocean basins being the aquatic organisms as the indicator species. PAHs and HPAHs entering the oceans through various sources can enrich lower trophic aquatic organisms and transfer them to the top predators in the food chain, so evaluation of toxic chemicals in edible aquatic species is important. The habitat and feeding information of the species were given in supplementary Table S2. The somatic body weights and fork lengths were recorded if the whole specimen was available. Samples were washed three times using distilled water and stored in an icebox at the collection site before being transported to the laboratory. At the laboratory, small to medium-sized specimens were processed by removing the head, skin, and viscera. For larger fish, approximately 200 g of muscle sample was taken from the anterior dorsal region, near to the pectorals. Samples were stored at less than −40 °C until sample preparation for analysis.

2.3. Sample preparation

The detailed sample extraction and cleanup methods for HPAHs and PAHs have been previously described elsewhere (Wickrama-Arachchige et al., 2020). Briefly, freeze-dried samples were precisely weighed and extracted using accelerated solvent extraction (ASE) apparatus. Then, one sample in each species was analyzed. Two surrogate standards (phenanthrene-d_{10} and perylene-d_{12}, 50 ng each) were spiked into the samples before extraction. The concentrated extract was purified using a silica gel column chromatograph and *n*-hexane. Further cleanup of the eluent was performed using gel permeation chromatography (GPC) connected to a column (EV-2000AC, Shodex, Japan). A mixture of cyclohexane and acetone (4:1 v/v) was used as the mobile phase of the GPC system at a flow rate of 5 mL/min. The representative fraction containing the target HPAHs and PAHs was collected from 15 to 55 min (approximately 200 mL). The GPC fraction was evaporated to a small volume and then taken to near-dryness under a nitrogen stream. Before

GC Orbitrap MS injection, fluoranthene-d_{10} (Flour-d_{10}, 50 ng) was added to the residue as the syringe spike. The entire extraction process was carried out under UV-protected conditions, with all glassware covered with aluminum foil to protect analytics from degradation. The GC/Orbitrap MS (Exactive GC system, Thermo Scientific, Waltham, MA, USA) was used to analyze HPAH and PAH quantities. The detailed analytical conditions needed for HPAHs and PAHs have been reported elsewhere (Wickrama-Arachchige et al., 2020).

2.4. Lipid content analysis

The lipid content of the dried samples was measured using a gravimetric method. Briefly, 2 g of the freeze-dried sample was precisely weighed, and lipids were extracted with *n*-hexane. A rotary evaporator was used to concentrate the lipids from the solvent extracts. Assuming that all lipophilic substances dissolved in the lipid, all HPAH and PAH data were converted to lipid base concentrations (ng/g lipid). Besides, the concentrations of target compounds were converted to ng/g dry weight (dw) for the comparisons of other studies, and the concentrations of the Sri Lankan samples were converted to ng/g wet weight for human health risk assessments.

2.5. Stable isotope analysis

Delipidated fish samples were used for the stable isotope analysis. Lipid removal was performed in a series of steps using an organic solvent. First, acetone was added to the sample, vigorously mixed, sonicated, centrifuged (12,000 rpm, 3 min), and the organic layer was removed. Second, chloroform with hexane was added, mixed well using a homogenizer (FastPrep-24, MP Biomedicals, Solon, OH) for sample preparation, centrifuged, and the organic layer was removed. The delipidation method was repeated three times for each sample. Samples were weighed (0.45 to 0.55 mg) in a tin capsule using an electronic balance and made into a pellet. Three pellets were made from one sample, which were then analyzed using a continuous-flow isotope ratio mass spectrometer (ANCA-GSL and Hydra 20–20, Sercon Ltd., UK) at the stable isotope facility of Meijo University, Japan. The mean values of stable isotopes were obtained from the analysis. Stable isotopic values are expressed in δ notation as parts per thousand (‰) deviation from the Pee Dee Belemnite for ^{13}C and atmospheric N_2 for ^{15}N (Fry, 2006).

$$\delta X = \left[\left(R_{sample} / R_{standard} \right) - 1 \right] \times 1000$$

where X is ^{13}C or ^{15}N and R is the isotopic value ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Generally, δ15 N can be used to determine the trophic position of an organism in a food web since there is on average a 3.4‰ increase of δ15N in consumers in one trophic level relative to the prey that they consumed in the other trophic level (Minagawa & Wada, 1984). δ13C can be used to identify a primary carbon source since there is about a 1‰ increase with each trophic level (Fry, 2006).

2.6. Calculation of the trophic magnification factor

Biomagnification potentials of ClPAHs, BrPAHs, and PAHs in edible aquatic species were determined by a trophic magnification factor (TMF). The TMF can be quantified with the equation derived from the concentrations of contaminants and tropic positions which are assessed through δ15N proxy (Nfon et al., 2009). Log concentration of total ClPAHs, total BrPAHs, and total PAHs (lipid normalized) as well as concentrations of each congener of HPAH and PAH, were examined against N15‰ for OPSL and CESL species. In this calculation, the TMF of each congener detected more than 3 samples were only considered for graphing regression line and the not detected samples were removed from the analysis. Linear regression with a trend line and equation was obtained from the following equation:

$$Log_{10}[\text{contaminant}] = a + b \times \ ^{15}N$$

The concentration variations of target compounds per changing unit of trophic positions is the slope (b) also named as biomagnification power (Nfon et al., 2009) and the constant (c) is the concentrations of background contaminants (Rolff, Broman, Näf, & Zebühr, 1993). The TMF can be quantified using the following formula (Nfon et al., 2009).

$$TMF = 10^{b}$$

The antilog of the slope is the trophic magnification factor (TMF). If the concentrations of target compounds increase with trophic level, the chemical is undergoing biomagnification where the TMFs are>1.0. Whereas, if the concentrations decrease with trophic level, the chemical is undergoing biodilution (i.e. bioreduction), where the TMF is less than 1.0 (Nfon et al., 2009).

2.7. Assessment of potential risks of consuming aquatic species to human health

The intake risks for dioxin-like HPAHs and PAHs were evaluated from toxic equivalents (TEQs), that is the sum of concentrations of each congener weighted by the potency of its corresponding relative toxicity. The estimation was given by the following equation:

$$TEQ = \sum (PAH_i \times TEF_i) + \sum (ClPAH_i + REP_i) + \sum (BrPAH_i + REP_i)$$

where *i* indicates i^{-th} congener. Note that the partial TEQs of PAHs were calculated using the toxic equivalency factor (TEF) based on the potency of BaP ($TEF_{BaP} = 1$), whereas the parts of HPAHs were calculated using the relative potency (REP) estimated from yeast AhR activity of each congener in place of the TEF (Ohura et al., 2007). The TEF or REP value of each congener used for the assessment is listed in Table S1. Also, the TEQs for the Indian Ocean samples have concentrations that were adjusted by both dry and wet weight, but only a dry weight basis was used for the Pacific Ocean samples.

The Incremental Lifetime Cancer Risk (ILCR) faced by senior adult Sri Lankans (males and females) was assessed. The consumption of fish and other aquatic species containing PAHs and HPAHs may increase the ILCR, which was calculated using the following equation (Wang et al., 2018; Xia et al., 2010; Yoon et al., 2007). The ILCR for humans from consuming aquatic species was assessed using the acceptable risk level (1×10^{-6}) and priority risk level (1×10^{-4}) (Boström et al., 2002; Wang et al., 2018; Xia et al., 2010).

$$ILCR = \sum TEQ \times IR_m \times ED_m \times SF / (BW_m \times AT_m)$$

where TEQ is the toxic equivalency quotient (mg/g wet wt) of PAHs or HPAHs; IR_m is the aquatic species intake rate (g wet wt/day) by a human for a selected age group *m*; ED is the exposure duration (years) for age group *m*; SF is the oral cancer slope factor [per (mg/kg)/day]. The SFs were obtained from the US-EPA Integrated Risk Information System (IRIS). The assigned SF was 7.3 for PAHs and HPAHs (Brune et al., 1981). BW is the average body weight (kg) of the age group *m* and AT is the average time (years) of the age group *m*. Due to a lack of age-related data for Sri Lanka, in this study, we focused on just one age group, i.e., adults whose maximum age was 70 years. The bodyweight of a senior adult person in Sri Lanka was set as 55 kg for males and 47 kg for females (Hewa Kuruppuge & Prasanna, 2013); the intake rate of aquatic biota per person was set as 43.2 g/day (MOFAR, 2016). The ILCR for each target compound was also separately estimated using TEQs related to those congeners and used for comparisons.

2.8. Statistical analysis

Linear regression was performed to separately study associations between the concentrations of total HPAHs and PAHs of aquatic species

from both geographic locations. Each dataset was assessed for homogeneity of variance and normal distribution before the parametric analysis, and the non-parametric Kruskal–Wallis, and Mann–Whitney U tests were used for any violations of parametric assumptions. For each analysis, the level of significance was set as $p < 0.05$. Moreover, the multivariate analysis of cluster variables was performed to visualize the distribution of congener profiles. MINITAB 19 was used to perform the statistical analyses. The graphs were prepared using Microsoft Excel 2019 and MINITAB 19 statistical software.

2.9. Quality control and quality assurance

For quality controls of the current study, the recovery of spiked surrogates was 48–88% for perylene-d_{12} and 43–69% for phenanthrene-d_{10} in all samples. The concentrations of targets are calculated from the standard solutions including the surrogate spikes so that the correction by recovery rates does not do to estimate the concentrations. Possible contamination was determined by running procedural blank samples at the beginning and then every ten samples, and at the last. The mean levels of blank for HPAHs and PAHs ranged from below method detection limits (MDL) to 0.49 ng (Cl_4Fluor), and from below MDL to 1.3 ng (Phe), respectively, and the concentrations of the target compounds were then calculated after deducting corresponding blank values. The MDL of targets ranged from 6.3 (9,10-Cl_2Phe) to 16 pg (ClPery), the details are represented elsewhere (Wickrama-Arachchige et al., 2020). For data below MDL, the concentrations were replaced with the value MDL/2.

3. Results and discussion

3.1. Concentrations of HPAHs and PAHs

Thirty ClPAHs, 21 Br PAHs, and 24 PAHs were investigated as contaminants in 39 edible aquatic organisms (38 different species) from three locations. From the 75 target compounds, 73 were detected in the samples, whereas 3,4-Cl_2Flu and 9-BrFl did not detect in any samples. The detection rate (%) and mean concentration of normalized lipid content (ng/g lipid) of individual targets for each of the sample locations are given in Table S1. In the Indian Ocean samples (OPSL and CESL), the detection rates tended to be higher than in the MJP samples, suggesting that the target pollutants accumulated more in the Indian Ocean samples than in the Pacific Ocean samples. In fact, the mean concentrations of ΣPAHs, ΣClPAHs, and ΣBrPAHs were 449, 84.4, and 20.3 ng/g lipid for OPSL; 379, 145, and 22.4 ng/g lipid for CESL; and 110, 20.4, and 2.99 ng/g lipid for MJP, respectively, demonstrating an accumulation order of ΣPAHs > ΣClPAHs > ΣBrPAHs in all locations, which was consistent with that previously reported for air and sediment samples (Ohura et al., 2015, 2009). In addition, the concentrations of each congener in the Indian Ocean samples (OPSL and CESL) were approximately ten times higher than the corresponding concentrations in MJP samples (Fig. 1). On the other hand, no statistically significant differences for any congener ($p > 0.05$) were observed between OPSL and CESL, even though the distance of these habitats from land is different. The spatial distribution variation of PAHs in fish indicated that the source of PAHs had a significant influence on PAHs contamination in the near-coastal region (Li et al., 2019). Therefore, higher accumulation of PAHs and HPAHs in the Indian Ocean samples could reflect the widespread and highly polluted status of the Indian Ocean.

To compare the results of the current study with those of other studies, the concentrations were standardized using the unit of ng/g-dw. The concentrations of ΣClPAHs ranged from 2.58 to 56.5 ng/g-dw in Sri Lankan samples and 0.35 to 18.3 ng/g-dw in Japanese samples (Table 1), which was considerably higher than those previously reported (Masuda et al., 2019; Tan et al., 2019). This could be due to the smaller number of target compounds in these earlier studies. To the best of our knowledge, this is the first study to investigate BrPAHs in various

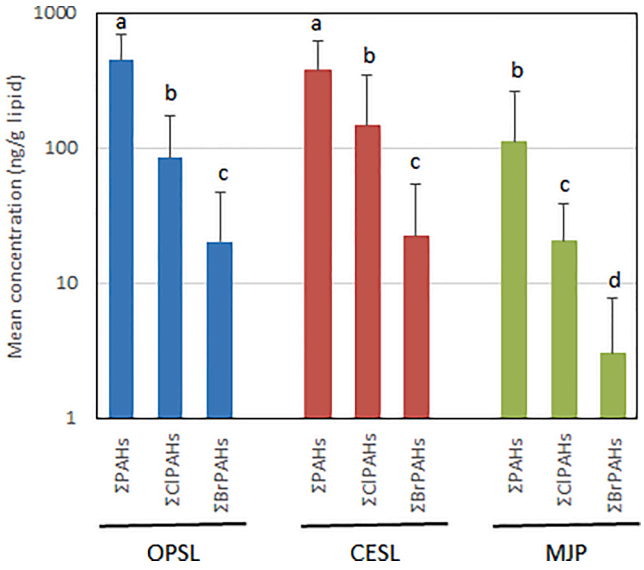

Fig. 1. Mean concentrations of ΣPAHs, ΣClPAHs, and ΣBrPAHs in OPSL, CESL, and MJP.

aquatic biological samples. The concentrations ranges of ΣPAHs in OPSL, CESL, and MJP were 27.5 to 117 ng/g-dw, 26.5 to 56.3 ng/g-dw, and 0.28 to 34.3 ng/g-dw, respectively (Table 1). The levels in the samples from the Indian Ocean were comparable to those reported in freshwater fish from the Liaoning China (Tan et al., 2019) but lower than levels reported in fish from the Persian Gulf (Jafarabadi et al., 2020), East China sea (Wang et al., 2019). For MJP, the concentration of ΣPAHs seemed to be distinguished at low level among aquatic samples in the East Asian area reported herein (Masuda et al., 2019; Moon et al., 2010; Tan et al., 2019; Li et al., 2019). According to Ackerman et al. (2008), atmospherically deposited organic contaminants, including PAHs, can accumulate at high levels in fish. Indeed, the order of magnitude of hydroxylated PAHs and PAH metabolites in urine from the general population of Asian countries showed a similar order of concentrations to that seen in fish: Japan < China < India < Korea < Kuwait (Guo et al., 2013). Therefore, terrestrial anthropogenic activities could trigger marine pollution in each area, followed by the accumulation of pollutants in fish.

We also investigated the relationships between PAHs and HPAHs in samples to evaluate the accumulation behavior of these compounds. Concentrations of PAHs and HPAHs in environmental samples such as air and sediments sometimes showed significant correlations (Ohura et al., 2015; Sun, Ni, & Zeng, 2011). However, the concentrations of ΣPAHs in our aquatic samples showed no significant correlations ($p > 0.05$) with either ΣClPAHs or ΣBrPAHs in any of the locations (Fig. S1) which did not cope with our hypothesis. It is suggested that certain PAHs are more susceptible to degradation in biota (Qin et al., 2020). Nevertheless, to date, there is no information on the biodegradation kinetics of HPAHs by metabolism and assimilation in biota. Although these behaviors of HPAHs remain unclear in aquatic species, our data implied that the efficacy of metabolism and assimilation of HPAHs was different from that of PAHs.

3.2. Comparison of contaminations among aquatic species

Comparing the concentrations of each congener among the samples in OPSL, the highest accumulator of ΣPAHs was *Selar crumenophthalmus* (901 ng/g lipid), for ΣClPAHs, the concentration was highest in *Tetrapturus audax* (399 ng/g lipid), and the highest ΣBrPAH concentrations was observed in *T. audax* (106 ng/g lipid) (Fig. S2). These results suggested that *T. audax*, which is a carnivore at a higher trophic level (Fig. S5), was one of the species at most concerned for accumulating

Table 1
Comparison of the total concentration range (ng/g dry weight) of PAHs and HPAHs in aquatic species at different geographic locations in Asia.

Location/sampling year	Origin of samples	Aquatic species or product (sample size = n)	ΣPAHs	ΣClPAHs	ΣBrPAHs	Reference
Sri Lanka/2018	Local market	Off-shore pelagic species (19)	27.5–117	2.58–27.1	0.30–9.53	This study
	Local market	Coastal and estuarine species (11)	26.5–56.3	3.87–56.5	0.44–8.51	This study
Japan/2018	Local market	Marine species (10)	0.28–34.3	0.35–18.3[a]	0.03–3.34[b]	This study
Japan/2017	Local market	Raw fish (2)	6.4	0.021		(Masuda et al., 2019)
Liaoning, China	Local fishery	Freshwater fish (22)	31.2–111	0.006–0.21[c]		(Tan et al., 2019)
South Korea/2005–2007	Local market	26 marine species as seafood (78)	12.3–243			(Moon et al., 2010)
South China/2016		Coral reef fish (21)	13–409			(Li et al., 2019)
East China Sea/2015	Wild	*Larimichthys crocea* (60)	267–695			(Wang et al., 2019)
Persian Gulf/2014	Wild	3 fish species (50)	634–1160			(Jafarabadi et al., 2020)

[a] : Eight out of 10 species detected.
[b] : Seven out of 10 species detected.
[c] : Three out of 22 species detected.

HPAHs.

Among the samples from CESL, *Lethrinus nebulosus* had the highest concentration of ΣPAHs, at 977 ng/g lipid, then the highest concentrations of ΣClPAHs was observed in *Scarus ghobban* (706 ng/g lipid), and the highest concentrations of ΣBrPAHs was observed in *S. ghobban* (106 ng/g lipid) (Fig. S2). In the coastal and estuarine species, *S. ghobban*, which feeds on benthic algae and corals and belonged to the middle trophic level (Fig. S5), could be one of the high accumulators of HPAHs.

In the MJP samples, *Gadus macrocephalus* had the highest ΣPAHs concentration (508 ng/g lipid), for ΣClPAHs, *Scomber japonicus* (54.7 ng/g lipid), had the highest concentrations than the other species, and *Patinopecten yessoensis* (13.0 ng/g lipid) showed the highest accumulations of ΣBrPAHs. Unlike, the Indian Ocean samples, the Pacific Ocean samples had three different species for the highest concentrations of each target compound. Consequently, the data revealed a vast species-specific discrepancy in HPAHs accumulation between these oceans, with elevated concentrations of target compounds in aquatic species that feed on small fishes and in benthic-feeding fish that consume benthic algae as well as plankton.

3.3. Profile of the congeners

When comparing the contributions of each congener that accumulated in the aquatic species, the profiles of individual ClPAHs and PAHs were quite similar in the OPSL and CESL except for a few organisms, but this was not the case for BrPAH congener patterns (Fig. S3). PAHs are ubiquitous and dominant, so they will contaminate not only the aquatic environment but also all types of prey consumed by aquatic species. Similarly, ClPAH congeners may also be omnipresent in these marine ecosystems. However, the distribution of BrPAHs in the aquatic environment may be different. In this study, we considered a range of aquatic species collected from Sri Lanka and Japan and those species had different feeding habits and different habitats, which may lead to differences in their exposure to HPAHs. These exhibited different compositions of congeners among the species and this may reflect different sources of the compounds. The three most dominant ClPAH congeners detected in all species analyzed were Cl_4Py, Cl_2Py, and 1-ClPy (Fig. S3), suggesting that they may be less metabolized in fish, irrespective of their presence in their food. The two most dominant BrPAH congeners detected in both the OPSL and CESL samples were similar: Br_4Py and 9-BrPhe. The third most dominant BrPAH congener detected in OPSL samples was 9,10-Br_2Ant, while in CESL it was 2-BrFl (Fig. S3). In contrast, the most dominant BrPAH congeners detected in MJP samples were α-BrNap, 9-BrAnt, and 9,10-Br_2Ant. This might reflect that the distribution of BrPAH in marine ecosystems has regional dissimilarities and/or the metabolic capacity could be species-specific (Fig. S3). The dominant PAH congeners detected in the muscles of all species tested in this study were Nap, Phe, and Py (Fig. S3). These data agreed with earlier published data for fish (Essumang et al., 2014; Wickrama-Arachchige et al., 2020) and suggests that these congeners are directly influenced by the higher trophic levels in aquatic media (Qin et al., 2020).

To determine any similarities in the abundance of congeners in the samples, we performed cluster analysis. The distance and similarity results indicated that 8 clusters were reasonably sufficient for the partition of congeners in all samples collected from Sri Lanka and Japan. The congeners of chlorinated pyrene and brominated BaA were grouped in cluster III which showed 65.2% similarity, and two highly abundant PAHs (Nap and Py) were grouped in cluster I showing 78.2% similarity (Fig. S4). These results indicated that highly abundant congeners were accumulated in these specimens in a similar way irrespective of the region that they were collected. Nevertheless, most of the carcinogenic PAHs (BaA, BaP, IP, BghiP) made another cluster II, while another carcinogenic PAHs (BbF, BjF + BkF, and Chry) made cluster V (Fig. S4). These findings suggested that each clustered compound could be identical accumulation properties in aquatic organisms.

3.4. Accumulation behaviors of HPAHs and PAHs in trophic levels

Stable isotopic nitrogen and carbon analysis were performed in the samples from the Indian Ocean (i.e., OPSL and CESL samples). The $\delta^{15}N$ and $\delta^{13}C$ values ranged from 13.70 to 8.38 and from −15.52 to −17.40 in the OPSL samples, and from 15.29 to 6.61 and from −14.31 to −27.41, in the CESL samples, respectively (Fig. S5). The trophic levels of a food web can be determined by $\delta^{15}N$ signatures (Jennings & Warr, 2003; Minagawa & Wada, 1984). Anthropogenic sources of nitrogen (e.g., sewage, fertilizers, and agricultural waste) and river run-off laden with inorganic nutrients close to CESL ecosystems may, in turn, cause the ^{15}N enrichment of particulate organic matter (McClelland et al., 1997), reflected by comparatively higher $\delta^{15}N$ values in the tissues of aquatic animals (Griffin & Valliela, 2001). The distance from the coast influenced both $\delta^{13}C$ and $\delta^{15}N$ values, indicating that there was spatial variability in ^{15}N and $\delta^{13}C$ values (Jennings & Warr, 2003). Nevertheless, salinity and temperature were correlated with base $\delta^{15}N$, and these physical parameters were more pronounced in coastal and estuarine ecosystems than the off-shore pelagic ecosystem, where temperature and salinity showed little variation (Jennings & Warr, 2003). It could be expected that the $\delta^{15}N$ source may differ between the OPSL and CESL ecosystems, and so we discussed the OPSL and CESL samples separately. The trophic level classification of organisms was described in Supplementary data in more detail.

Next, we compared the mean concentrations of ΣPAHs, ΣClPAHs, and ΣBrPAHs in the aquatic organisms based on their categorized trophic levels. For the OPSL samples, the total concentrations of all congeners increased with increasing trophic levels (Fig S6). This trend was particularly prominent in ΣClPAHs and ΣBrPAHs, although there were no significant differences ($p > 0.05$). In addition, the contribution of relatively high molecular-weight HPAHs, such as Cl_4Py and Br_4Py, increased with increasing trophic levels (Figure S6). This suggested that such HPAHs might be resistant to being metabolized in organisms, so

they accumulated as the trophic level increases. On the other hand, such elevations in concentrations were not observed in each trophic level in CESL (Fig. S7). Overall, the contribution profiles of each congener except for those HPAHs with relatively high molecular weights were to some extent consistent throughout the trophic levels in both aquatic areas. These findings indicated that the processes of metabolism and/or accumulation of PAHs and HPAHs were common throughout the trophic levels, whereas the contaminant levels of habitat ranged in the aquatic environment could be mainly contributed to the accumulation. There may be several reasons for this observation. One may be the difference in availability of target compounds between the CESL and OPSL environments. It seems reasonable to expect comparatively higher concentrations of HPAHs in the CESL organisms than the OPSL organisms due to HPAHs potentially being more readily available from point sources on or near land. Another reason may be the selective feeding of top carnivores residing in the CESL environment. The concentrations of analytics in the open ocean food chain may be lower due to the increased distance from land-based point sources. Consequently, there may be fewer HAPHs in the prey of the middle trophic level. Another possible reason could be the age of the species in the higher trophic level, as well as their feeding habits and their habitat (Table S2). We previously noted that the concentration of HPAHs and PAHs increases with increasing body size (body weight) and age of tuna (Wickrama-Arachchige et al., 2020). In this study, there were two species of non-mature individuals, *Ephinephelus malabaricus* and *Silago sihama*, in the higher trophic level in the CESL samples. Although the stable isotope study showed that they belong to the higher trophic level, they contained lower concentrations of target compounds compared with the concentrations in other species. It can be expected that fish with a long lifespan could ingest large amounts of bioaccumulative toxic compounds from their diet and also from the ambient environment through direct contact, with these compounds eventually accumulating in their tissues. The degree of HPAHs absorbance via the body contact and gill filtration of fish is not known.

Aquatic biota may be unintentionally exposed through bioconcentration to these target compounds, which may be attached to floating or submerged organic matrixes and associated with their food and surrounding water and/or sediments. Elimination rates, reduced bioavailability, and variable uptake of contaminated food could be the several factors for the bioaccumulation of toxic chemicals in fish (Jafarabadi et al., 2020). It can be assumed that most of their diet is contaminated with PAHs and HPAHs to varying degrees, via bioconcentration, biomagnification, and therefore bioaccumulation. For instance, it was noted that some planktonic and benthic feeders among the OPSL species, such as *S. crumenophthalmus,* and *Stolephorus indicus*, had higher levels of PAHs (Fig. S5, Table S2). Furthermore, *S. ghobban*, which feeds on benthic algae and corals, showed the highest total concentrations of ClPAHs and BrPAHs in the CESL group (Fig. S5). These species had higher levels of accumulation, similar to those seen in large, predatory fishes. This finding indicated that there was a yet unknown mechanism(s) by which HPAHs and PAHs enter marine biota, other than the dietary route through bioaccumulation, and it would be direct contact with the environment and unintentional ingestion through opportunistic feeding.

3.5. Trophic magnification factor of aquatic species

The TMF tool was applied to evaluate bioaccumulation of total ClPAHs, total BrPAHs, and total PAHs in aquatic species collected from OPSL and CESL and compared TMF for each congener of PAH, ClPAH, and BrPAH. There was a positive relationship between log concentration of total ClPAHs, total BrPAHs, and total PAHs (lipid normalized), and N15‰ for both OPSL and CESL organisms (Fig. 2). The analysis revealed that the TMF is above 1.0 for total concentrations of PAHs, ClPAHs, and BrPAHs which showed biomagnification of target compounds in the edible aquatic organisms collected from the above ecosystems (Table S3). These findings support our hypothesis that HPAHs may poses similar biomagnification tendencies in biota as per PAHs. In particular, the biomagnification properties of HPAHs were enhanced in OPSL than in CESL (Fig. 3). This could be due to the differences in feeding habitat of aquatic species living in those environments as described above.

Furthermore, congener-specific TMF showed that most of the congeners of HPAH and PAH biomagnified in the aquatic edible species both in OPSL and CESL environments (Fig. 3). The detailed information of TMF calculation for each compound is represented in Table S3. There were 95% of congeners (detected and the sample size is more than 3 for

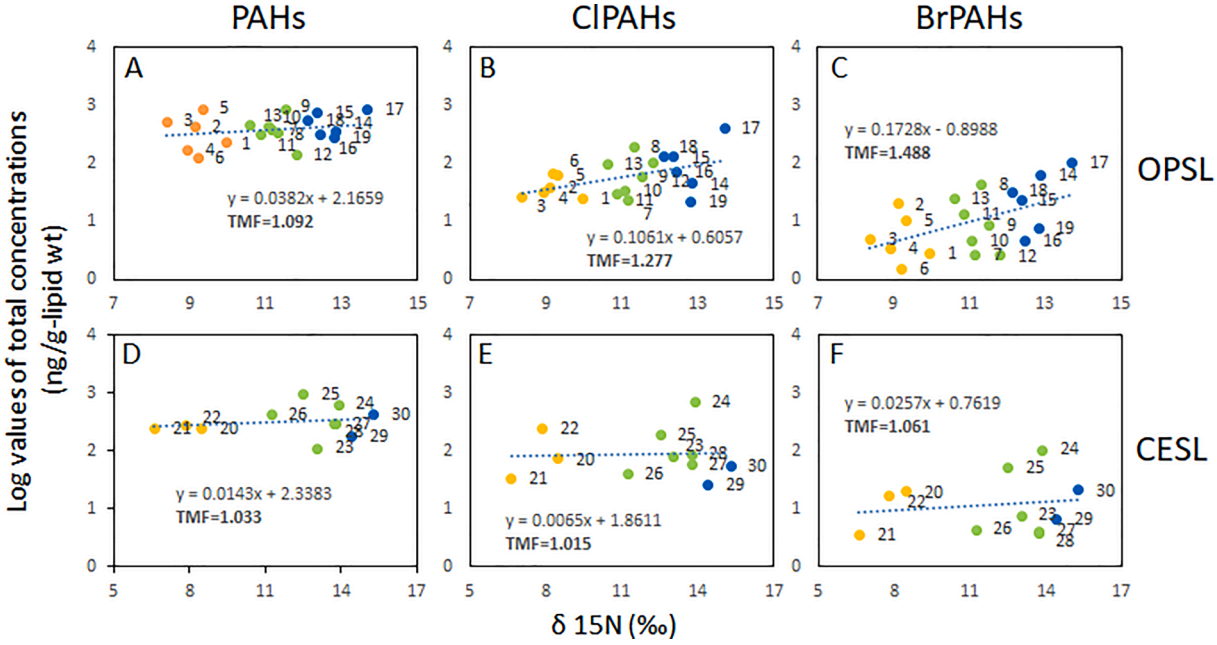

Fig. 2. Relationships between δ15N (‰) and total concentrations of (A, D) PAHs, (B, E) ClPAHs, and (C, F) BrPAHs (ng/g-lipid wt) in aquatic species of (A–C) OPSL and (D–F) CESL. The index numbers of plots correspond to those in Table S2. Orange, green, and blue circles represent aquatic species of lower, middle, and higher trophic level, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

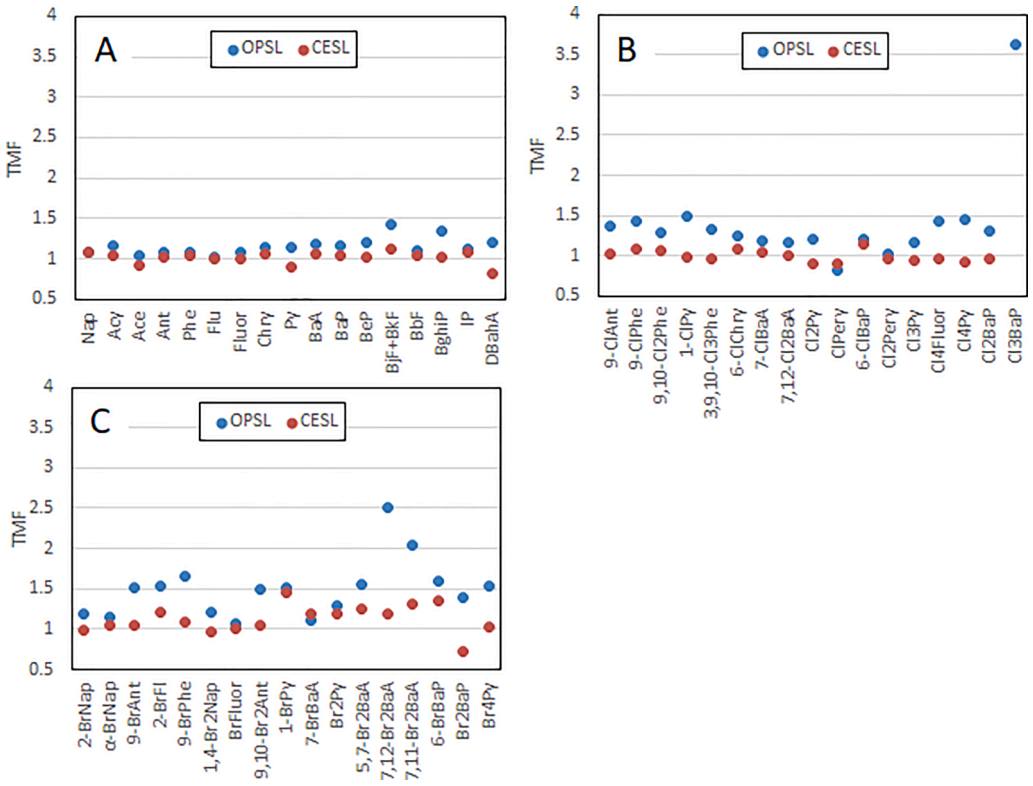

Fig. 3. TMF of individual compound of (A) PAHs, (B) ClPAHs and (C) BrPAHs as the congeners.

regression analysis) for PAHs, 88% of congeners for ClPAHs, and 100% of congeners in BrPAHs showed TMF higher than 1 indicating their biomagnification potentials for OPSL organisms. In addition to that, there were 84% of congeners for PAHs, 56% of congeners for ClPAHs, and 79% of congeners for BrPAHs showed TMF higher than 1 indicating biomagnification potentials for CESL organisms. Among PAHs, combined concentrations of BjF and BkF showed the highest biomagnification potentials both for OPSL and CESL organisms (Fig. 3A). For the ClPAHs, the highest TMF was obtained from Cl_3BaP for OPSL organisms and 6-ClBaP for CESL organisms (Fig. 3B). Collectively congeners of chlorinated pyrene were prominent in the biomagnification process. For the BrPAHs, 7,12-Br_2BaA showed the highest TMF for OPSL organisms while 1-BrPy was the congener showed the highest TMF for CESL organisms (Fig. 3C). Overall, halogenated pyrenes were the leading congeners that biomagnified in the samples.

3.6. Human health risk assessment of consuming aquatic species

To assess the risk to human health, we first categorized PAHs assessed into three groups: 24 congeners of PAH (∑24PAHs), 16 congeners of US-EPA priority PAHs (∑16PAHs), and 7 congeners of carcinogenic PAHs (∑7cPAHs) (Table S4). From the studied PAHs in the Indian Ocean samples, the occurrence of US-EPA priority 16 PAHs (∑16PAHs) was over 99%, and carcinogenic PAHs (∑7cPAHs) was below 7%. The higher occurrence rate of ∑7cPAHs to ∑24PAHs was observed from *Risoprionodon acutus* (7.04%), *Mobula kuhlii* (6.70%), and *Loligo duvauceli* (5.40%) and their respective ∑7cPAHs concentrations were 1.12, 0.59, and 0.74 ng/g wet wt (Table S4). The species containing the highest concentration of ∑7cPAHs in Sri Lanka included top carnivores, small piscivores, and planktivores which further give the insight to explore trophic level accumulation differences.

Next, we compared the TEQ concentrations, normalized by the dry weight of the samples, from each location. The mean TEQ concentrations estimated from all corresponding congeners (total TEQs) in OPSL, CESL, and MJP were 0.72, 0.80, and 0.27 ng/g dw, respectively (Fig. S8). For the contribution of each congener, the magnitude order, exception for CESL, showed a similar trend of raw concentrations: PAHs > ClPAHs > BrPAHs, whereas ClPAHs were the highest congeners in CESL (Fig. S8). This difference in CESL could be due to high levels of ClPAH contamination in *Oreochromis niloticus* and *Arius caelatus* (Fig. S5), suggesting that these species may be linked to an increase in the risk to human health.

Furthermore, we evaluated the ILCR induced by exposure to PAHs and HPAHs via fish consumption for male and female senior adults in Sri Lanka, with the TEQs normalized to the wet weight of the samples. The mean values of the ILCR estimated from all target congeners (calculated by using TEQs of HPAHs and PAHs) in OPSL and CESL were 7.6×10^{-7}

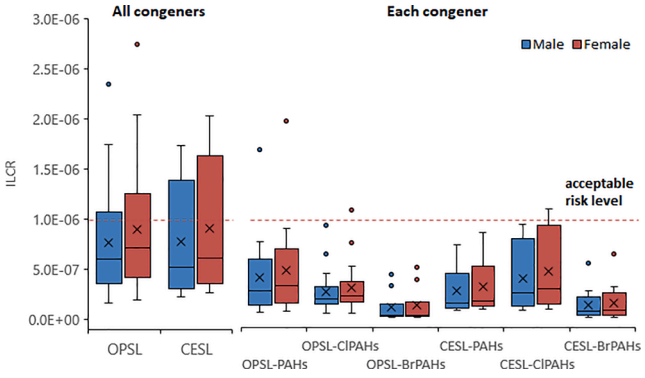

Fig. 4. The incremental lifetime cancer risk for males and females by consumption of aquatic species in different locations and congeners. The central boxes represent values from the lower to upper quartiles (25th to 75th percentiles). T = minimum and maximum. The cross mark and middle line represent the mean and median values, respectively. The red dotted line represents the US-EPA acceptable level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and 7.8×10^{-7} for males and 8.9×10^{-7} and 9.1×10^{-7} for females, respectively, therefore slightly higher for females than for males (Fig. 4). The mean values were slightly lower than the acceptable risk level suggested by the US-EPA (1×10^{-6}). However, approximately one-third of the species exceeded the acceptable risk level (i.e. 5 and 7 out of 19 OPSL samples exceeded the acceptable risk level for male and female senior adults, respectively, and it was 4 out of 11 for the CESL samples for both genders) (Table S5). The organisms that exceeded the acceptable risk level in the OPSL were *R. acutus*, *M. kuhlii*, *L. duvauceli*, *Acanthocybium solandri*, and *T. audax*. The organisms that exceeded the acceptable risk level in CESL were *O. niloticus*, *S. ghobban*, *Penaeus monodon*, and *A. caelatus*. According to US-EPA, the ILCR above acceptable risk level indicated that a one in a million chance of additional human cancer over a 70-year lifetime by consuming above aquatic species (US-EPA, 2000). Besides, the current values were higher than those reported for the South China Sea ($1.6 \times 10^{-8} - 2.2 \times 10^{-7}$) (Yu et al., 2019) and lower than those reported for coral reef fish in the Persian Gulf (ILCR $> 1 \times 10^{-5}$) (Jafarabadi et al., 2020). Comparing the contributions of each congener to the ILCR, PAHs and ClPAHs formed the major contribution in the OPSL and CESL samples, respectively, although their levels were considerably lower than the acceptable risk level (Fig. 4). These findings indicated that not only PAHs but also ClPAHs are of concern as a risk to human health through the consumption of certain aquatic species. The acceptable risk level of PAHs in *R. acutus* in OPSL was slightly exceeded for both males (1.6×10^{-6}) and females (2×10^{-6}) while the risk level of ClPAHs in *M. kuhlii* (1.1×10^{-6}) was exceeded for females. In CESL, *A. caelatus* (1.1×10^{-6}) and *P. monodon* (1.1×10^{-6}) slightly exceeded the acceptable risk levels of ClPAHs for females. Notably, the levels of PAHs and ClPAHs in fish were enhanced when they were grilled (Masuda et al., 2019). Therefore, further exploration of variations in contaminants between raw and cooked products will be important to evaluate the cancer risks for consumers.

4. Conclusions

This study investigated the presence of 75 congeners of HPAH and PAH in 39 different edible aquatic species in the Indian Ocean and the Pacific Ocean, resulted that 73 congeners were detected in the samples. Among the targets, halogenated pyrenes were predominant in the samples. Overall, the aquatic species containing higher levels of the targets exhibited a variety of food and feeding habits, indicating that there are exposure routes that can lead to the accumulation of HPAHs and PAHs in the tissues of aquatic organisms. Further, the overall mean values of HPAHs and PAHs between the trophic levels clearly showed possible biomagnification (TMF > 1) of contaminants in the Indian Ocean samples. Human health risk assessment revealed that consumption of some aquatic species such as *R. acutus*, *M. kuhlii*, *L. duvauceli*, *A. solandri*, *T. audax*. *O. niloticus*, *S. ghobban*, *P. monodon*, and *A. caelatus* in the Indian Ocean region might present some health risk concerns for senior adults in Sri Lanka, although the mean acceptable risk level for all species did not exceed the acceptable risk level. Finally, we can conclude that both oceans have deteriorated considerably as a result of next-generation persistent organic pollutants, such as HPAHs, which enter aquatic species and ultimately accumulate in humans through the dietary consumption. Therefore, it is essential to maintain a healthy ocean by minimizing anthropogenic ocean pollution, to ensure a better future for humans.

Contributors

Anura Upasanta-Kumara Wickrama-Arachchige: Investigation, Formal analysis, Data curation, Writing - original draft. **Keerthi S. Guruge:** Project administration, Supervision, Data curation, Writing - review & editing. **Yuriko Inagaki:** Investigation, Formal analysis, Data curation. **Hinako Tani:** Investigation, Formal analysis, Data curation. **Tilak Siri Dharmaratne:** Supervision, Resources. **Yasuaki Niizuma:** Resources. **Takeshi Ohura:** Project administration, Methodology, Supervision, Data analysis, Writing - review & editing, Funding acquisition.

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.

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Appendix A. Supplementary data

Concentrations of individual congeners at each location, detail information of aquatic species, TEQ concentrations of each aquatic species, Details of TMF, total concentrations of all PAHs, US-EPA priority PAHs, and 7 of the carcinogenic PAHs, ILCR for Sri Lankan senior adults who consume aquatic species, relationships of concentrations between ΣPAHs and ΣHPAHs, concentrations of ΣPAHs, ΣClPAHs, and ΣBrPAHs in individual aquatic organisms, compositions of individual congeners in each aquatic species, trophic levels and composition of congeners in trophic levels of Indian Ocean samples, and TEQ concentrations of each congener in each location. Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.130072.

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