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# Occurrence of polycyclic aromatic hydrocarbons (PAHs) in coral reef fish from the South China Sea

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ABSTRACT

Little data are available on the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in coral reef fish from the South China Sea (SCS). In this study, we collected 21 coral reef fish species from the Xisha and Nansha Islands in the SCS to investigate the occurrence of 16 US-EPA PAHs. The total PAH concentrations (ΣPAH) in the collected fish ranged from 12.79 to 409.28 ng/g dry weight (dw, Xisha Islands) and from 32.71 to 139.09 ng/g dw (Nansha Islands), respectively. The ΣPAH concentration of *Scarus niger* collected from the Xisha Islands (237.13 ng/g dw) was about twofold higher than that of *Scarus niger* collected from the Nansha Islands (139.09 ng/g dw). The dominant compounds were found to be 2-ring and 3-ring PAHs. Based on qualitative and quantitative analyses, the main PAH sources were found to be coal and biomass combustion (50.43%), petroleum sources (25.86%), and vehicular emissions (16.10%).

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of persistent organic pollutants (POPs) that contain at least 2 fused aromatic rings. PAHs are widespread environmental contaminants in the global biogeochemical cycle due to their persistence, long-range transport, and bioaccumulation (Ko et al., 2014; Burns and Jones, 2016; Sun et al., 2016; Ranjbar Jafarabadi et al., 2017; Wang et al., 2017a, 2017b). To date, > 200 PAHs have been detected in the environment, many of which have been identified as carcinogenic, mutagenic, and teratogenic (Kim et al., 2013; Ko et al., 2014; Gu et al., 2016; Wang et al., 2017a, 2017b), with 16 being listed as priority pollutants by the US Environmental Protection Agency (US-EPA). Although PAHs can originate from natural sources such as volcanoes and forest fires, the main sources of PAH released into the environment are anthropogenic activities (Ko et al., 2014; Burns and Jones, 2016; Ranjbar Jafarabadi et al., 2017).

In marine environments, PAHs can be rapidly adsorbed by suspended particulate matter due.

to their hydrophobic properties. They can then be deposited in sediment or taken up and bioaccumulated by marine biota (Bandowe et al., 2014; Ko et al., 2014; Sun et al., 2016), and so can eventually affect the health of human populations who consume seafood products (Bandowe et al., 2014; Sun et al., 2016). Indeed, the accumulation of PAHs in marine organisms poses a long-term burden on biogeochemical cycling through food chains in the ecosphere (Ko et al., 2017), with the marine environment being regarded as a sink for PAHs (Bandowe et al., 2014; Sun et al., 2016; Wang et al., 2016). Therefore, the trophic transfer of PAHs in aquatic ecosystems must be examined to assess the ecological risk of these chemicals in marine ecosystems.

Coral reefs, ecologically critical areas with high biodiversity and productivity (Clark, 1995), provide a myriad of ecosystem services to humans (Eyre et al., 2014) and support a high diversity of fish that depend on the corals for their survival (i.e., coral reef fish). However, this specific shallow water ecosystem is sensitive to human impact, with anthropogenic activities having an increasingly serious impact in recent decades (Fabricius, 2005; Hughes et al., 2013; Hughes et al., 2017), including bleaching and a decline in reef-dwelling organisms (Gardner et al., 2003; Wilson et al., 2006; Yu, 2012; Perry et al., 2013; Zhao et al., 2016a, 2016b). It has therefore been considered that the levels and distributions of POPs in coral reef biota are vital criteria for assessing the ecological risk of anthropogenic activities on coral reef systems (Ko et al., 2014). In this context, POPs such as PAHs have been detected in coastal coral reef biota, including legacy POPs such as polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and its

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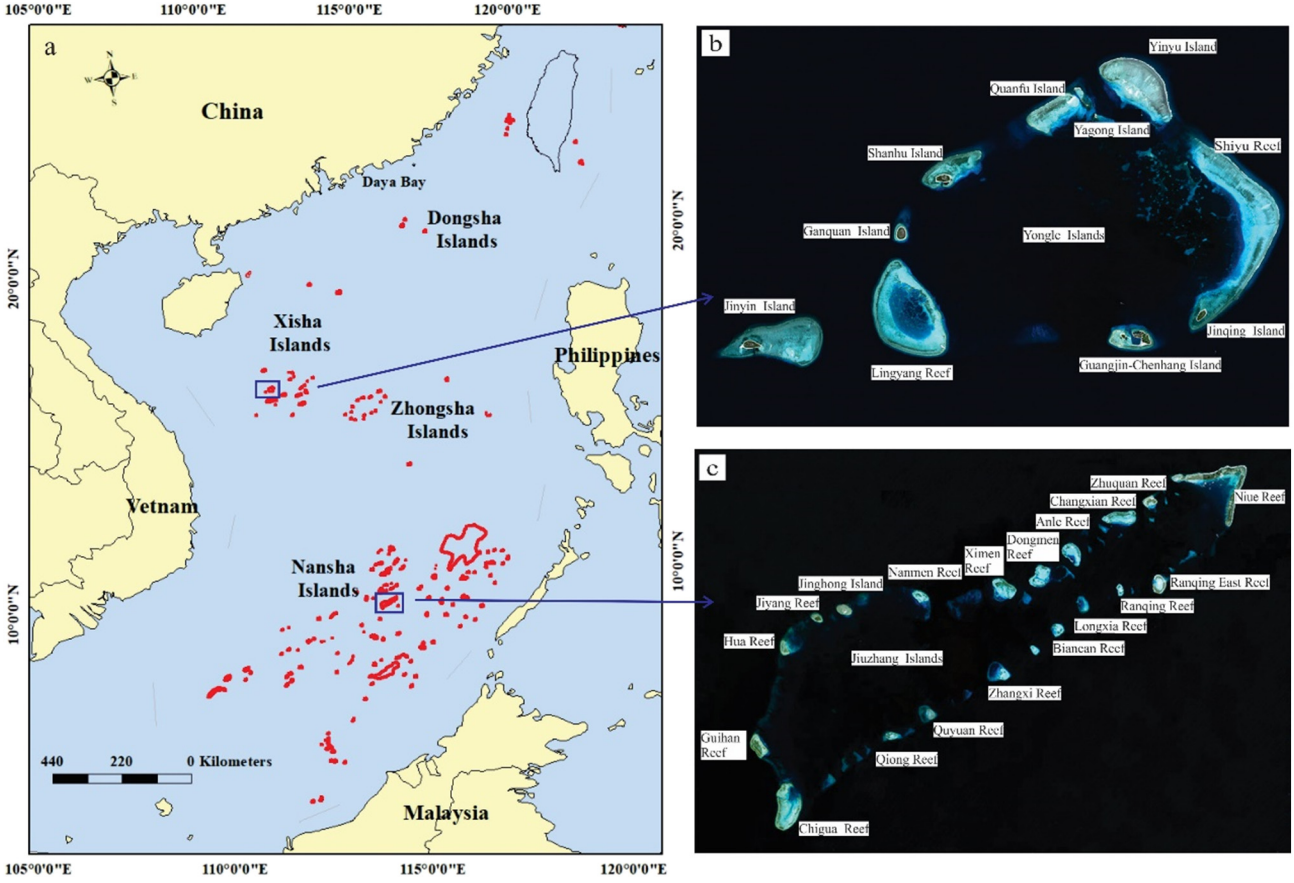


**Fig. 1.** Study area: (a) general location of the Xisha and Nansha Islands, (b) detail of Yongle Atoll, (c) detail of Jiuzhang Atoll.

metabolites (Hao et al., 2014; Ko et al., 2014; Sun et al., 2014; Sun et al., 2017).

The South China Sea (SCS), with an area of 350 million km$^2$, is the third largest marginal sea in the world, and is located at the center of the Indo-West Pacific biogeographic province (Morton and Blackmore, 2001). Coral reefs, as the only shallow water marine habit in offshore areas of the SCS, are mainly distributed in the Dongsha, Xisha, Zhongsha, and Nansha Islands (Fig. 1). Adjacent to the western border of the "Coral Triangle", the ecological and economic significances of the coral reefs of the SCS have long been emphasized (Burke et al., 2002; Zhao et al., 2016a, 2016b; Yu, 2012). As the SCS is surrounded by several developing countries such as China, the Philippines, Malaysia, and Vietnam, rapid population and economic growth in these countries have contributed to the increased surface runoff and atmospheric deposition of POPs in the areas of the SCS (Hao et al., 2014; Sun et al., 2016; Sun et al., 2017). In addition, the SCS is a choke point connecting the Indian and Pacific Oceans, and so oil spills from shipwrecks and oil tankers also contribute to PAHs release (Morton and Blackmore, 2001). However, the SCS is a traditional fishery zone for surrounding countries (Zhang et al., 2016) with an estimated sustainable fish catch of > 2 million tons per year (http://www.hinews.cn/news/system/2012/08/07/014756317.shtml). Although 3000 fish species were recently identified in the SCS (Randall and Lim, 2000) with > 500 of these species being coral reef fish (http://www.hinews.cn/news/system/2012/08/07/014756317.shtml), the presence of PAH contaminants in coral reef fish from the SCS has yet to be examined.

Although recent studies have examined the PAH contamination status in northern parts of the SCS in terms of source identification, transformation, and risk assessment in coral tissues, marine fish, and sediment (Yang, 2000; Luo et al., 2004; Huang et al., 2012; Ko et al., 2014; Ke et al., 2017), little attention has been paid to coral reef fish in offshore areas of the SCS. Indeed, the PAH levels of coral reef fish in central and southern areas of the SCS have yet to be investigated due to their significant distance from the mainland. However, details regarding the PAH levels and concentrations in coral reef fish would be expected to provide key data for understanding the contamination degree of the coral reef system in the SCS. Indeed, such contaminant inventories would be expected to be of critical importance for future studies into trophic transfer and bioaccumulation in coral reef ecosystems, in addition to aiding in decision making related to the ecological restoration of coral ecosystems. Thus, here we report the collection of 21 species of coral fish samples from the Xisha and Nansha Islands of the SCS and the subsequent analysis of these samples for the presence of 16 PAHs. More specifically, we investigate the PAH levels and composition profiles in coral reef fish from central and southern parts of the SCS and assess the possible sources of these PAHs. Finally, we compare and discuss interspecies differences and their implications on the coral reef fish from the Xisha and Nansha Islands.

## 2. Materials and methods

### 2.1. Study area and sample collection

Coral reef fish species were sampled from Yongle Atoll (15°46′–17°07′N and 111°11′–112°03′E) in the Xisha Islands and Jiuzhang Atoll (9°42′–10°00′ N, 114°15′–114°40′E) in the Nansha Islands, in the SCS in December 2016.

#### 2.1.1. Yongle Atoll

The Xisha Islands are located southeast of the continental shelf and of Hainan Island, in approximately the central part of the SCS (Fig. 1). The Yongle Atoll, which has a total area of 350 km$^2$, is the largest atoll

**Table 1**
Characteristics, feeding habits, and locations of coral reef fish sampled in this study.

| Sample ID | Species | Lipid (%) | Body length (cm) | Feeding habit | Location |
|---|---|---|---|---|---|
| 1 | *Epinephelus quoyanus* | 0.106 | 17 | Invertebrate feeder and piscivore | Xisha Island |
| 2 | *Paracaesio sordida* | 0.049 | 34 | Planktivore | Xisha Island |
| 3 | *Lethrinus erythropterus* | 0.026 | 30 | Invertebrate feeder | Xisha Island |
| 4 | *Lethrinus lentjan* | 0.030 | 28 | Invertebrate feeder and piscivore | Xisha Island |
| 5 | *Plectorhinchus chaetodonoides* | 0.024 | 33 | Invertebrate feeder and piscivore | Xisha Island |
| 6 | *Lutjanus gibbus* | 0.023 | 23 | Invertebrate feeder | Xisha Island |
| 7 | *Macolor niger* | 0.053 | 33 | Invertebrate feeder and piscivore | Xisha Island |
| 8 | *Scarus ghobban* | 0.042 | 29 | Herbivore | Xisha Island |
| 9 | *Scarus forsteni* | 0.029 | 27 | Herbivore | Xisha Island |
| 10 | *Scarus forsteni* | 0.022 | 30 | Herbivore | Xisha Island |
| 11 | *Scarus oviceps* | 0.040 | 33 | Herbivore | Xisha Island |
| 12 | *Scarus niger* | 0.063 | 30 | Omnivore | Xisha Island |
| 13 | *Scarus rivulatus* | 0.018 | 29 | Herbivore | Xisha Island |
| 14 | *Cheilinus fasciatus* | 0.020 | 30 | Invertebrate feeder | Xisha Island |
| 15 | *Cephalopholis argus* | 0.040 | 23 | Invertebrate feeder | Xisha Island |
| 16 | *Myripristis berndti* | 0.031 | 20 | Invertebrate feeder | Xisha Island |
| 17 | *Plectorhinchus diagrammus* | 0.025 | 34 | Invertebrate feeder | Xisha Island |
| 18 | *Navodon tessellates* | 0.030 | 25 | Planktivore | Nansha Island |
| 19 | *Pterocaesio tile* | 0.130 | 16 | Planktivore | Nansha Island |
| 20 | *Scarus niger* | 0.063 | 24 | Omnivore | Nansha Island |
| 21 | *Balistapus undulatus* | 0.173 | 22 | Invertebrate feeder and piscivore | Nansha Island |
| 22 | *Scomberomorus sinensis* | 0.007 | 26 | Invertebrate feeder and piscivore | Nansha Island |
| 23 | *Scomberomorus sinensis* | 0.009 | 70 | Invertebrate feeder and piscivore | Nansha Island |
| 24 | *Scomberomorus sinensis* | 0.064 | 140 | Invertebrate feeder and piscivore | Nansha Island |

of the Xisha Islands and is located in the southwest region. It comprises Ganquan Island, Shanhu Island, Quanfu Island, Yagong Island, Yinyu Island, Shiyu Reef, Jinqing Island, Guangjin–Chenhang Island, Jinyin Island, and Lingyang Reef (Fig. 1b). Among these islands, the Yagong, Yinyu, and Quanfu Islands are open to tourists. Yongle Atoll experiences the East Asian Monsoon climate with abundant rainfall, high humidity levels, high temperatures, and an average year-round temperature of 26.5 °C.

#### 2.1.2. Jiuzhang Atoll

The Nansha Islands are located in the southern part of the SCS (Fig. 1). All coral islands and reefs lie scattered over an area of ~820,000 $km^2$. Jiuzhang Atoll is located in the northwestern part of the Nansha Islands, and has a total area of 558 $km^2$, which is comprised of > 20 islands and reefs (Fig. 1c). Jiuzhang Atoll is subject to the tropical marine monsoon climate influenced by the northeast monsoon in winter and the southwest monsoon in summer. The average year-round temperature is ~28.1 °C (Yu et al., 2006).

#### 2.1.3. Sample collection

Twenty-one coral reef fish species (24 samples in total, Table 1) were collected from coral reef areas of the Xisha and Nasha Islands in the SCS. Among them, 16 fish species (17 samples, *Scarus forsteni*, n = 2) were collected in Yongle Atoll, Xisha Island, while 5 species (7 samples, *Scomberomorus sinensis*, n = 3) were collected from Jiuzhang Atoll of the Nansha Islands. All fish samples were obtained with the aid of hired local fishermen from Tanmen by diving and collecting on-site. These coral reef fish were selected because they are reef dwellers and are therefore susceptible to bioaccumulation of pollutants. The concentration of pollutants in these species acts as a good indicator of the pollution level of coral reef ecosystem. Most of the coral fish were economic species and collected from different positions of the two atolls. To get a comprehensive understanding of the pollution level of different coral reef fish species living the reef environment, fish species with roughly similar body length were selected. Once on the deck, the fish samples were placed directly in a freezer at −20 °C prior to transportation to the laboratory. All fish species were identified at the Nantong Aquatic Products Research Institute. The samples were rinsed three times using ultrapure water, and their body lengths were measured, as outlined in Table 1. The dorsal muscles were excised from the fish, dried by vacuum freeze-drying, then ground into a fine power and stored at −20 °C prior to analysis.

### 2.2. Sample preparation and analysis

A sample of dried fish (5 g) was spiked with surrogate standards including naphthalene-$d_8$ (Nap-$D_8$), acenaphthene-$d_{10}$ (Ace-$D_{10}$), phenanthrene-$d_{10}$ (Phe-$D_{10}$), chrysene-$d_{12}$ (Chr-$D_{12}$), and perylene-$d_{12}$ (Pre-$D_{12}$) prior to extraction with dichloromethane over 48 h using Soxhlet apparatus. After this time, an aliquot of the extract was used for analysis of the lipid content by heating at 65 °C for 30 min prior to weighing. Another aliquot of the extract was purified by gel permeation chromatography using a glass column packed with SX-3 Bio-Beads (40 g, Bio-Rad Laboratories, USA) and eluted with dichloromethane/*n*-hexane (v/v = 1:1). The PAH-containing eluate (90–280 mL) was collected and concentrated to a volume of 1 mL prior to further purification on a multilayer column packed with 3 cm of neutral alumina, 3 cm of silica gel, and 1 cm of anhydrous sodium sulfate (from bottom to top) and eluted using dichloromethane/*n*-hexane (50 mL, v/v = 1:1). The elute was then concentrated to near dryness under $N_2$ and reconstituted in *n*-hexane (100 μL).

Sixteen priority PAHs, including naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorine (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flo), pyrene (Pyr), benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), indeno[1,2,3-c,d]pyrene (IndP), dibenzo[*a,h*]anthracene (DahA), and benzo[*g,h,i*]perylene (BghiP) were analyzed by gas chromatography/triple quadrupole mass spectrometry (GC–MS/MS, Thermo Fisher Scientific, TSQ 8000 Evo, USA) using a quartz capillary TG-5MS column (30 m × 0.25 mm × 0.25 μm film thickness). Helium was used as the carrier gas with a flow rate of 1 mL/min. The GC temperature program was as follows: initial temperature of 70 °C (hold for 1 min), heat to 140 °C at a rate of 25 °C/min, heat to 240 °C at a rate of 10 °C/min, then heat to 300 °C at a rate of 5 °C/min and hold for 4 min. The temperatures of the injection and ion sources were 270 and 280 °C, respectively. All samples were analyzed in the selected reaction monitoring (SRM) mode.

### 2.3. Quality assurance/quality control

Analyses of the procedural blanks, spiked samples, and duplicate samples (n = 2) were carried out with each sample set (12 samples) to ensure quality control. Quantification was performed by the external standard method (Wang et al., 2015) using a reference standard containing 16 PAHs at 9 standard concentrations (1, 10, 50, 100, 200, 400, 500, 800, and 1000 ng/L), and the obtained correlation coefficients for all calibration curves were > 0.999. Recoveries of the surrogates generally ranged from 50% (Nap-$D_8$) to 126% (Chr-$D_{12}$) of the spiked concentrations. The concentrations of the PAH compounds were not corrected by the surrogate recoveries. The relative percentage analysis differences between duplicate samples was < 15. Procedural blank analysis showed no detectable amounts of PAHs.

### 2.4. Data analysis

Since the analysis results of the duplicate samples presented only minor differences (< 15%), the original data for the PAHs compounds were used in the results. Two source identification methods, namely molecular diagnostic ratios (MDRs) and principal component analysis (PCA), were employed to identify the possible sources of PAHs, along with specific conger concentration ratios of Ant/(Ant + Phe) and BaA/(BaA + Chr). PCA combined with multiple linear regression (MLR) analyses was used to analyze the data sets of the PAH compound profiles to quantify the possible sources and their respective contributions. A more detailed description of the PCA/MLR method can be found in the literature (Sofowote et al., 2008; Wang et al., 2016). PCA/MLR analysis was conducted using SPSS 24.0 software.

## 3. Results and discussion

### 3.1. PAHs levels and interspecies differences in the coral reef fish samples obtained from the Xisha and Nansha Islands

The concentrations of the PAHs in the coral fish obtained from Xisha Island ranged from 12.79 ng/g dry weight (dw) in *Lethrinus erythropterus* to 409.28 ng/g dw in *Cephalopholis argus* (Fig. 2a), with an average concentration of 71.69 ng/g dw. Indeed, Fig. 2a shows that clear interspecies differences were present (standard deviation, SD = 103.8). Among the various species examined, the PAH concentrations in *Cephalopholis argus*, *Scarus niger*, and *Lethrinus lentjan* were significantly higher than those present in other species (Fig. 2a), likely due to their trophic level and feeding habits. In addition, Fig. 2b shows the calculated results of the PAH levels in coral fish from the Nansha Islands. In this case, the concentrations ranged from 32.71 ng/g dw in *Pterocaesio tile* to 139.09 ng/g dw in *Scarus niger* (SD = 37.2), and the average concentration was 67.69 ng/g dw, slightly lower than that of the Xisha Island fish samples. Although previous studies have suggested that PAH levels among different fish species may be influenced by a range of factors such as the lipid content (Sun et al., 2016), no significant relationship was observed in our study. Furthermore, the benthic species were expected to have a high PAH burden due to their increased contact with sediment, which readily adsorbs POPs, as indicated above (Sun et al., 2016; Sun et al., 2017). As the coral reef area is a shallow water ecosystem, there were no obvious differences in the living habits, and so we considered that feeding habits were most likely responsible for the varying PAH concentrations in the different fish species.

Based on a previous report (Gao et al., 2014) and trophic information from FishBase (https://www.fishbase.de/), five feeding habits were classified for all the coral fish species in this study (planktivore, omnivore, invertebrate feeder, invertebrate feeder and piscivore, and herbivore). The coral fish with higher PAHs concentration were invertebrate feeders and piscivores, omnivores, and invertebrate feeders. These species occupied higher trophic levels in the food chain of the coral reef ecosystem, suggesting that the feeding habits of different coral reef fish species have important influences on the bioaccumulation of PAHs and that coral fish located at higher trophic levels contain elevated levels of PAHs concentration.

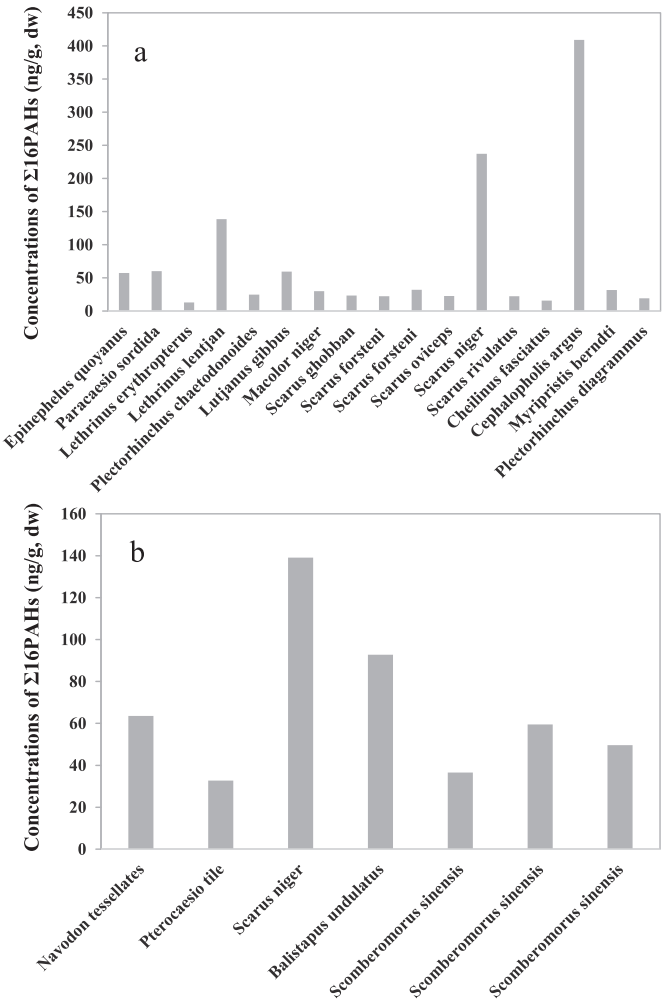


**Fig. 2.** Concentrations of the total PAH contents in coral fish obtained from (a) the Xisha Islands and (b) the Nansha Islands in the SCS.

Among all species, *Scarus niger* (which feeds on coral and invertebrates) was found in both the Xisha and the Nansha Islands, and this species contained PAH concentrations of 237.13 and 139.09 ng/g dw, respectively. This significant difference for the same species with same trophic level and feeding habits indicated that the PAH level in coral fish could be used as indicator to imply the environment quality of coral reef systems. In contrast to the significant interspecies concentration difference, a smaller intraspecies variability was present in *Scarus forsteni* with concentrations of 22.34 ng/g dw (Sample Number 9) and 31.99 ng/g dw (Sample Number 10) being determined for Xisha Island samples. A similarly small variability was observed for *Comberomorus sinensis*, where concentrations of 36.60, 59.55, and 49.62 ng/g dw were detected for samples 22–24 from the Nansha Islands, respectively.

To the best of our knowledge, this is the first study investigating the PAHs present in coral reef fish obtained from the SCS, although several POPs have recently been studied in marine organisms from coral tissues located at coastal regions of the SCS (Hao et al., 2014; Ko et al., 2014; Sun et al., 2014; Sun et al., 2017). Thus, Table 2 summarizes the PAH concentrations in the marine biota of the SCS and of other locations. As indicated, the PAH levels of coral reef fish reported herein were significantly lower than those of the marine fish obtained from Daya Bay

**Table 2**
Comparison of PAH concentrations (ng/g, dry weight) in marine biota from the SCS with those of other locations.

| Medium/species no. | Sampling location | Region | Sampling year | No. of PAHs | Concentration (ng/g; dry weight) | Reference |
|---|---|---|---|---|---|---|
| Marine fish/19 | Daya Bay | Northern coastal of SCS | 2012 | 16 | 440–2080[a] | Sun et al., 2016 |
| Marine organisms/15 | South China Sea | North of SCS | 2015 | 16 | 94.88–557.87(289.86)[b] | Ke et al., 2017 |
| Coral fish/16 | Yongle Atoll, Xisha Islands | Central of SCS | 2016 | 16 | 12.79–409.28 (71.69)[b] | This study |
| Coral fish/5 | Jiuzhang Atoll, Nansha Islands | Southern of SCS | 2016 | 16 | 32.71–139.09 (67.69)[b] | This study |
| Coral tissues/2 | Kenting National Park, Taiwan | Northern of SCS | 2009–2010 | 16 | 143–1715 | Ko et al., 2014 |
| Marine fish/3 | Coast of Ghana | West Africa | 2010 | 28 | 71–481(192)[b] | Bandowe et al., 2014 |
| Marine organisms/278 | Gulf of Mexico | USA | 2010–2011 | 25 | 30–210 | Xia et al., 2012 |
| Notothenioid fish/6 | Eastern Antarctica | Antarctic | 2009 | 24 | 38–107.2[a] | Ko et al., 2018 |

[a] Data reported based on wet weight basis for comparison using a wet/dry ratio of 4 (Ke et al., 2017).
[b] Mean value of PAHs concentration.

(520–2080 ng/g dw, Sun et al., 2016) and from northern regions of the SCS (289.86 ng/g dw, Ke et al., 2017), and an obvious decrease in the PAH burden of fish samples was observed in southern areas of the SCS compared to those detected in northern areas (Table 2). It should be noted that the spatial distribution variation of PAH levels in fish samples indicates that the PAH source has a significant influence on PAH contamination in the near coastal region. In addition, the PAH concentrations of the 16 PAHs present in coral reef fish from the SCS were comparable to those in marine organisms from the Gulf of Mexico (USA) and East Antarctica (Xia et al., 2012; Ko et al., 2018; Table 2), while lower than concentrations of coral tissues detected in Kenting National Park, Taiwan (Ko et al., 2014).

As PAHs can transfer along the food chain, the PAH data obtained from coral reef fish could be employed to evaluate the influence of anthropogenic activities on coral reef ecosystems. As such, the pollutant concentrations of these marine biotas provide a good indication of the levels of pollution in the waters, sediments, and coral tissues of the coral reef. Furthermore, Ke et al. (2017) suggested that the consumption of marine organisms from the northern part of the SCS posed a negligible risk to human health. The PAH concentration levels found in this study are lower than those from the northern part of SCS, which indicates that health risks arising from the consumption of the studied fish are minimal.

### 3.2. Compositional patterns of the PAHs

All 16 PAHs were detected in the fish samples collected from coral reef areas in central (Xisha Islands) and southern (Nansha Islands) parts of the SCS, and the compositional patterns of the PAHs based on their number of aromatic rings are provided in Fig. 3. As indicated, the relative compositions of the PAHs were comparable, and low molecular

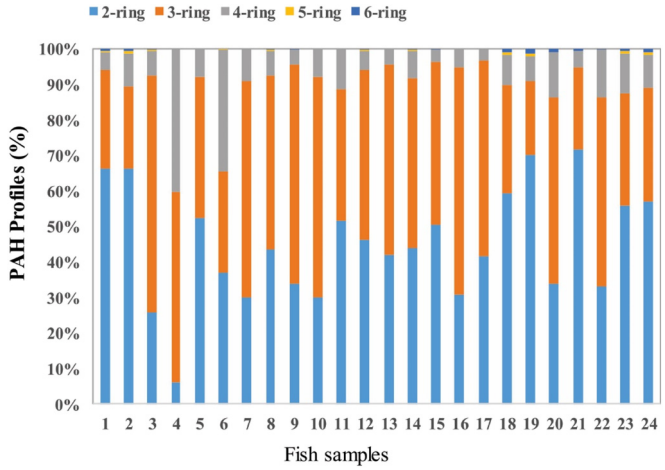


**Fig. 3.** PAH profiles (aromatic ring number) for the coral fish collected from the SCS (Xisha Islands samples 1–17, Nansha Islands samples 18–24).

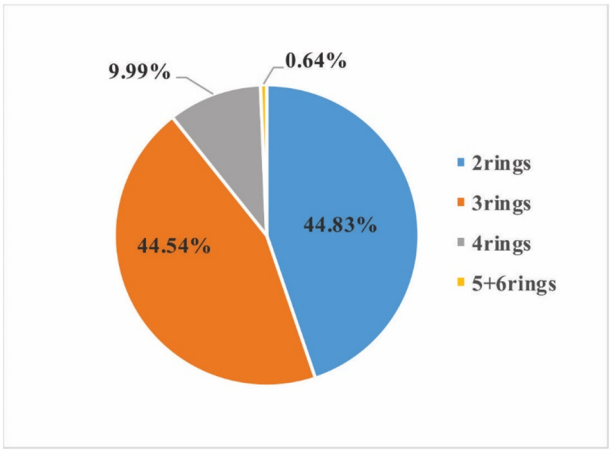


**Fig. 4.** Relative compositions of the 16 PAHs in coral fish from the SCS.

weight PAHs (LMW = 2–3 rings) dominated in the fish muscles, accounting for 59.60–96.60% of the total content. In the present study, 2- and 3-ring PAHs composed approximately 44.83 and 44.54% of the total, followed by 4-ring and 5 + 6-ring PAHs, which accounted separately for 9.99 and 0.64% of the total content (Fig. 4). In addition, Nap, Phe, and Flu were found to be the major compounds, accounting for 44.83, 18.55, and 12.21% of the total content, respectively. Furthermore, no significant compositional profile differences were observed between coral reef fish from the Xisha and Nansha Islands. This distribution pattern was consistent with previous studies into marine fish from the northern part of the SCS (Sun et al., 2016; Ke et al., 2017) and for the compositional pattern of coral tissues and coral sediment from Kenting Park, located in the northern region of the SCS (Ko et al., 2014). The reef sediment PAH pattern reported in the Persian Gulf of Iran was also dominated by 2–3 ringed LMW-PAHs, which accounted for ~62% of the total PAHs. These consistent patterns among coral reef fish, coral tissues, and surficial coral sediments from other locations of the world suggest that PAH pollution transfers via a specific mechanism in the coral reef ecosystem. Further studies are required to investigate this mechanism further.

### 3.3. Source Identification

#### 3.3.1. Molecular diagnostic ratios

The molecular ratios of PAHs are commonly employed as a qualitative source identification method to distinguish between combustion and petroleum sources (Yunker et al., 2002; Wang et al., 2016; Ke et al., 2017). Among these molecular ratios, Flo/(Flo + Pyr), Ant/(Ant + Phe), IcdP/(IcdP + BghiP), and BaA/(BaA + Chr) have been used to determine the possible sources of PAHs (Yunker et al., 2002; Wang et al., 2016; Ke et al., 2017). It should be noted that to distinguish between combustion and petroleum sources, Ant/(Ant + Phe) was proven to be

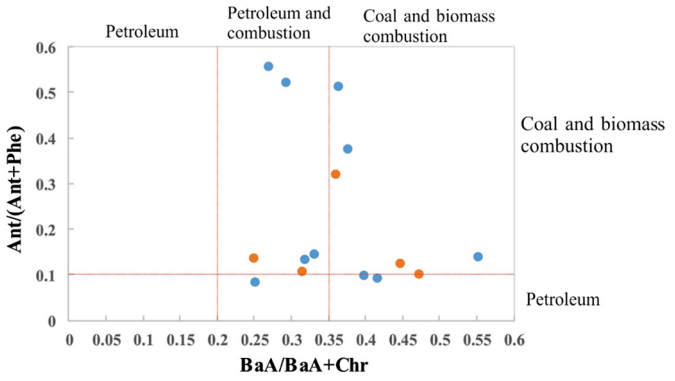


**Fig. 5.** PAH cross plots for the Ant/(Ant + Phe) versus BaA/(BaA + Chr) ratios in the coral fish samples obtained from the Xisha and Nansha Islands of the SCS.

more definitive than Flo/(Flo + Pyr) (Yunker et al., 2002). As IcdP was not detected in the majority of fish samples examined herein, Ant/(Ant + Phe) and BaA/(BaA + Chr) were used as indicators to identify the possible PAH sources in this study (Fig. 5).

As indicated in Fig. 5, no obvious differences were observed for the Ant/(Ant + Phe) and BaA/(BaA + Chr) ratios in the coral fish samples collected from the Xisha and Nansha Islands. More specifically, the majority of fish samples (i.e., 17 of the 20 samples) exhibited high values of Ant/(Ant + Phe) (> 0.1), which suggested that the combustion of coal, grass, and wood was a likely source of the PAHs in our study area. In addition, all BaA/(BaA + Chr) ratios were > 0.2, and approximately half of these were > 0.35, thereby indicating that a mixture of coal and biomass combustion in addition to petroleum were the main sources of the PAHs.

### *3.3.2. Principal component analysis with multiple linear regression analysis (PCA/MLR)*

As the MDR method is limited in calculating the contributions of different sources, a combination of PCA and MLR was employed as a quantitative method to identify the possible PAH sources in the coral fish. As no obvious differences in the molecular ratios and compositional patterns were observed in the fish obtained from the Xisha and Nansha Islands, it appeared that the PAH sources were likely similar, and so all samples were combined for PCA/MLR analysis.

Thus, the PCA analysis results for the PAH concentrations are presented in Table 3. As indicated, four principal components (PCs) were extracted for the coral reef fish samples, and the explained variances of the four factors were 30.49, 29.50, 23.04, and 8.94%, respectively, which accounted for 91.98% of the total variability. More specifically, PC1 was heavily weighted by Phe, Ant, DahA, and moderate loadings of Nap, BbF, and BaP. Thus, Phe and Ant were identified as components of wood combustion (Harrison et al., 1996; Larsen and Baker, 2003), while Bap was associated with coal combustion (Kavouras et al., 2001). PC1 was therefore considered to be derived from a mixture of coal and biomass combustion. In addition, PC2 was predominately weighted by Acy, Ace, and Flu, which are low molecular weight components containing three aromatic rings, and originating mainly from petroleum (Ko et al., 2014; Sun et al., 2016). Furthermore, PC3 was heavily loaded in Pyr, BaA and Chr. Since BaA was associated with gasoline combustion (Sofowote et al., 2008; Wang et al., 2016), and Chr was identified as a tracer of diesel vehicle emissions, PC3 was interpreted to represent loadings from vehicular emission sources including gasoline and diesel combustion. However, PC4 was unassigned due to insufficient chemical information to allow accurate source interpretation. Indeed, the existence of a fourth source is doubtful, as this source may arise from the inability of PCA to correctly scale the data prior to analysis. It was therefore apparent that in general, the PAHs present in coral reef fish obtained from the SCS originated from a mixture of biomass combustion, petroleum, and vehicular emissions.

**Table 3**
Varimax-rotated component loadings of the four principal components (PCs) for the PAH compositions of the coral fish obtained from the SCS.

| PAHs | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Nap | **0.813** | 0.491 | 0.001 | 0.189 |
| Acy | 0.234 | **0.921** | −0.066 | 0.071 |
| Ace | 0.352 | **0.898** | 0.055 | 0.043 |
| Flu | 0.321 | **0.868** | 0.055 | −0.018 |
| Phe | **0.846** | 0.341 | 0.352 | 0.082 |
| Ant | **0.892** | 0.251 | 0.294 | 0.010 |
| Flo | 0.512 | 0.391 | **0.710** | 0.184 |
| Pyr | 0.102 | −0.058 | **0.986** | 0.060 |
| BaA | 0.028 | −0.056 | **0.989** | 0.042 |
| Chr | 0.065 | −0.014 | **0.978** | 0.032 |
| BbF | **0.735** | 0.595 | −0.032 | −0.068 |
| BkF | 0.297 | 0.659 | −0.026 | 0.552 |
| BaP | **0.704** | 0.630 | 0.141 | 0.059 |
| IndP | −0.047 | −0.047 | −0.164 | **−0.889** |
| DahA | **0.927** | 0.211 | −0.042 | 0.164 |
| BghiP | 0.400 | **0.707** | −0.070 | 0.460 |
| Expl.var./% | 30.49 | 29.50 | 23.04 | 8.94 |

Note: PCA loading value higher than 0.7 is marked in bold.

The contributions of these three sources were then evaluated by MLR. Thus, coal and biomass combustion, representing 50.43% of the PAH sources, was common in this area due to the industrial and agricultural activities of surrounding developing countries (Morton and Blackmore, 2001; Hao et al., 2014; Sun et al., 2016; Sun et al., 2017). The second major contribution (i.e., 25.86%) was from petroleum sources resulting from oil spills in the SCS, while vehicular emissions from shipping and fishing activities contributed to 16.10% of the PAH content.

This contamination pattern in coral fish from the central and southern parts of the SCS was consistent with those reported for marine organisms in the northern part of the SCS (Ke et al., 2017). In addition, it was previously reported that PAHs of petroleum origin accumulated in the coral tissue of Kenting National Park, Taiwan (Ko et al., 2014). Overall, the PCA results were consistent with those obtained using the MDR method for identification of wood and biomass combustion and petroleum as PAH sources. However, the PCA method also identified a new additional source, thereby indicating that this method provided more detailed source information in addition to validating the source information given by the MDR method. Furthermore, the combined PCA/MLR method could quantitatively calculate the contributions of the various sources to the total PAH content and provides reliable results regarding source identification.

## 4. Conclusion

We investigated 16 polycyclic aromatic hydrocarbons (PAHs) that bioaccumulated in coral reef fish species collected from the Xisha and Nansha Islands of the South China Sea (SCS). Our results indicated that the levels of PAHs present in the coral reef fish samples were at the lower end of the whole SCS range, thereby suggesting a lower pollution degree of coral reef systems than of coastal areas in the SCS. In addition, the PAH compositional patterns were dominated by 2- and 3-ring PAHs, which indicated that the bioaccumulation of low molecular weight PAHs was common in these fish. Furthermore, two source identification methods (i.e., molecular diagnostic ratios and a combination of principal component analysis and multiple linear regression) were used to qualitatively and quantitatively characterize the PAH sources, and the results indicated that the PAHs present in coral reef fish originated mainly from coal and biomass combustion (50.43%), followed by petroleum sources (25.86%), and vehicular emissions (16.10%). This is the first report on levels of PAHs in coral reef fish in the offshore coral reef areas of the SCS, providing baseline data for further understanding of trophic transfer and bioaccumulation of POPs in coral reef ecosystems.

However, further studies are required to evaluate the fate and transfer mechanisms of PAHs in the whole coral reef ecosystem for effective environmental management in the region.

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