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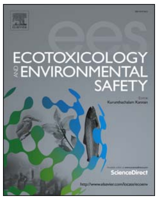

Occurrence and distribution of antibiotics in mariculture farms, estuaries and the coast of the Beibu Gulf, China: Bioconcentration and diet safety of seafood

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ABSTRACT

The occurrence, distribution, bioconcentration and diet safety via seafood consumption of 19 antibiotics were investigated in eight closed mariculture ponds, four estuaries, two nearshore areas and one offshore area from the Beibu Gulf. Seventeen, 16, 15 and 7 antibiotics were detected at total concentrations of 43.2 − 885 ng L^{-1}, 22.4 − 118 ng L^{-1}, 22.7 − 24.5 ng L^{-1}, and 1.81–3.23 ng L^{-1} in the water of the above different areas, respectively. This indicates that the mariculture ponds are important sources of antibiotic pollution on the coast of the Beibu Gulf. Ten antibiotics were detected in feed samples with concentrations ranging from 0.03 to 95.4 ng g^{-1}, demonstrating the presence of antibiotics in the feed and/or residual antibiotics in the raw material of the feed. The field bioconcentration factors (BCFs) of the antibiotics calculated in different culture organisms ranged from 0.55 to 10,774 L kg^{-1}. The estimated daily intakes (EDIs) of sulphonamides, fluoroquinolones, macrolides and chloramphenicols via aquatic products were 19.8–105, 33.7–178, 34.9–186 and 6.9–37.1 ng d^{-1}, respectively. According to the acceptable daily intakes (ADIs) and maximum residue limits (MRLs) proposed by different organisations, these aquatic products (shrimp, crab and oyster) reached the standard of safe consumption and could not pose a health risk to humans. However, a potential elevated risk to humans may remain because of the occurrence of multiple antibiotics in the cultured organisms, particularly for sensitive populations, such as pregnant women, the elderly and children.

1. Introduction

Antibiotics, as a type of effective antimicrobial agent, are widely used for bacterial disease prophylaxis and treatment in humans and animals and also as feed additives to promote growth in husbandry and aquaculture. China leads the world in antibiotic production and consumption (Chen et al., 2015a; Luo et al., 2011). It has been estimated that the annual production of antibiotics is approximately 248,000 t, of which 90% is used for humans (48%) and to treat animals (42%), and the remaining 10% is exported (Chen et al., 2015a). In recent years, the residues of antibiotics have been frequently found in water (Ma et al., 2015; Schwartz et al., 2003; Xu et al., 2007; Yan et al., 2013; Zhang et al., 2012a), sediments (Zhou et al., 2016), soils (Martinez-Carballo et al., 2007), suspended particles (Zhang et al., 2017; Zhou et al., 2013), faeces (Zhou et al., 2013)and biota samples (Chen et al., 2015b; Li et al., 2012). Antibiotics can cause toxic effects to aquatic and terrestrial organisms (Kim et al., 2007; Kotzerke et al., 2008) and can also bioaccumulate in organism. In addition, their continuous application can promote antibiotic resistance genes (ARGs) in bacterial populations (Eguchi et al., 2004; Su et al., 2012).

Various antibiotics are widely used in aquaculture to prevent disease, improve feed utilization and promote animal growth. However, only 20~30% is absorbed and utilized by aquaculture products. The remaining antibiotics persist in the aquatic environment and are discharged into the surrounding water, or they are deposited in the sediments of aquaculture ponds (Brooks et al., 2005; Mimeault et al., 2005; Samueisen, 1989; Schwaiger et al., 2004). This not only affects aquatic ecological environment but also leads to their concentrations in aquatic products over national and international food safety standards. Therefore, antibiotic residues in the aquaculture environment should be paid much attention due to their potential risk to human consumer.

The Beibu Gulf, located in the northwest of the South China Sea, is

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surrounded by Leizhou Peninsula, Qiongzhou Strait, Hainan Island, Vietnam and Guangxi Zhuang Autonomous Region. It covers an area of 1.3×10^5 km^2 and is a semi-closed gulf with an average depth of approximately 38 m (Chen et al., 2009). The climate around the gulf is subtropical and monsoonal (Chen et al., 2009). As an important mariculture base, the Beibu Gulf is one of the four famous fishing grounds in China. With a wide shallow sea and mudflat, it is suitable for the breeding and growth of multiple marine organisms. The mariculture area and production of Guangxi Province in 2012 were 530 km^2 and 977 thousand tons, respectively (Huang, 2013). The catch in the Beibu Gulf reached 857 thousand tons in 2012 (Zou et al., 2013). There are two main culture types based on different food sources: (1) fish, shrimp and crab culture, which depend on artificial feed and are usually cultured in closed high level ponds; and (2) shellfish culture, which ingest natural phytoplankton and are usually cultured in open estuaries and shallow seas. The fish, shrimp and crab culture area was 227 km^2, and production was 254 thousand tons. The shellfish culture area was 303 km^2, and production was 723 thousand tons (Huang, 2013). Organic matter and nutrients can be discharged into the gulf with water flow. Rapidly developing mariculture in the Beibu Gulf has caused marine antibiotic pollution. A previous study in 2010 showed that certain antibiotics were detected in seawater from the coast, estuary and the vicinity of an aquaculture farm in the gulf, with the highest concentration ranging from 0.53 to 51 ng L^{-1} (Zheng et al., 2012). Moreover, a higher intensity of aquaculture activities could contribute to increasing levels of antibiotics in the environment (Zheng et al., 2012). However, that study only referred to a few seawater samples in the vicinity of mariculture farms. Questions about the occurrence, distribution, bioaccumulation, food exposure risks and environmental impact of antibiotics in mariculture farms in a typical subtropical gulf were not resolved. Therefore, a comprehensive study is needed to resolve these questions. The objectives of this study are to: (1) select two typical culture models (high-place culture ponds and bamboo raft culture in an estuary) and natural sea areas (estuaries, nearshore and offshore areas); (2) investigate the occurrence and distribution of 19 antibiotics belonging to four classes among water, sediment, marine product and feed samples in mariculture farms on the marine environment; (3) calculate the bioaccumulation factors of antibiotics in different culture organisms; (4) estimate daily intakes of the marine products and evaluate the human risk of dietary exposure.

2. Materials and methods

2.1. Standards and reagents

The 19 target compounds belong to the following four different antibacterial groups: 1) 8 sulphonamides (SAs), including sulfacetamide (SAAM), sulfadiazine (SDZ), sulfamethoxazole (SMX), sulfathiazole (STZ), sulfamethazine (SMZ), sulfapyridine (SPD), sulfadimethoxine (SDM), and trimethoprim (TMP); 2) 5 fluoroquinolones (FQs), including norfloxacin (NOX), ciprofloxacin (CIX), enrofluxacin (ENR), ofloxacin (OFX), and enoxacin (ENX); 3) 4 macrolides (MLs), including erythromycin (ETM), clarithromycin (CTM), azithromycin (AZM), and roxithromycin (RTM); and 4) 2 chloramphenicols (CAPs), including florfenicol (FF) and chloramphenicol (CAP). The 19 high purity standards noted above and 4 isotope-labelled compounds used as surrogate standards ($^{13}C_3$-Caffeine, ^{13}C, D_3- ETM, $^{13}C_6$-SMX, D_5-NOX) were purchased from different manufacturers (Supplementary Table S1). Additionally, information on standards and reagents is summarised in the Supplementary information S1.

2.2. Sample collection

Seventeen sampling sites were investigated in this study (Fig. 1), including seven high-place shrimp culture ponds (01 P, 02 P, 04 P, 05 P, 07 P, 10 P and 12 P), one mudskipper culture pond (13 P), four estuaries (06E, 08E, 09E and 11E), two nearshore sites (03 N and 14 N), and three offshore sites (15 O, 16 O and 17 O) near Weizhou Island. All shrimp ponds cultured *Litopenaeus vannamei*, the 01 P, 04 P and 07 P ponds polycultured crabs (*Scylla paramamosain*). The estuaries are main oyster (*Crassostrea rivularis Gould*) culture areas. The offshore sites were selected as background. All samples (water, sediment, marine product and feed) were collected from the 17 sites in October 2015. Additionally, relevant sampling information is given in Tables S2 and S3.

All 17 water samples were collected using a stainless-steel bucket and were immediately poured into a 1-litre pre-cleaned amber glass bottle. Twelve sediment samples, fifteen biota samples and three feed samples were collected into sealed polyethylene bags. The detailed information on the sampling sites for sediment, biota and feed is summarised in Supplementary Table S2. All samples were stored at 4 °C during transport to the laboratory. The water samples were processed within 48 h. The weight and length of each biota sample were recorded. Then, the shrimp and oyster muscles were dissected with medical operation scissors and homogenized with agate mortar. The crab samples were divided into three parts (CL: crab leg muscle, CP: crab pereion muscle, and CR: crab roe) and homogenized. The sediment samples were freeze dried using freeze drier, ground and homogenized with agate mortar before passing through 80 mesh stainless steel sieve. All prepared biota and sediment samples were stored at −20 °C in a refrigerator prior to extraction.

2.3. Sample extraction and instrumental analysis

The extraction and instrumental analysis of water, sediment, feed and biota samples was performed as previously described (Chen et al., 2015b; Xu et al., 2007; Zhou et al., 2012). The water sample (1 L) was extracted with an Oasis hydrophile-lipophile balance (HLB) cartridge (6 mL, 500 mg), whereas the sediment and feed (5 g dry weight) were extracted by ultrasonic-assisted extraction with acetonitrile and citric acid buffer ($pH = 3$), followed by an enrichment and clean-up step with solid-phase extraction using strong anion exchange-HLB (SAX-HLB) cartridges in tandem. The biota samples (5 g wet weight) were extracted with methanol/water-0.1 M acetic acid (50:50, v/v) by ultrasonication with SAX/PSA-HLB cartridges in tandem. Additional details are shown in Supplementary S2.

Samples were analysed using an ultra-performance liquid chromatography–electrospray-ionisation tandem mass spectrometry (UPLC–ESI-MS–MS, Agilent UPLC 1290 tandem 6460 QQQ) with multiple-reaction monitoring (MRM). Separation of the target compounds was achieved on an Agilent Zorbax RRHD Eclipse Plus C18 column (2.1 mm × 100 mm, 1.8 µm) at 40 °C. Methanol (B) and high-purity water containing 5 mmol L^{-1} ammonium acetate aqueous solution with 0.1% formic acid (A) were the mobile phases, and the injection volume was 5.0 µL. A binary gradient at a flow rate of 0.3 mL/min was applied. The mobile gradient is shown in Table S5.

2.4. Quality assurance and quality control

A quantitative analysis of each compound was performed using UPLC-ESI-MS-MS with MRM mode using one or two of the highest characteristic precursor ion/product ion transitions. Together with the retention times, the characteristic ions were used to ensure correct peak assignment and peak purity. A known amount of $^{13}C_3$-CAF, ^{13}C,D_3-ETM, $^{13}C_6$- SMX and D_5- NOX were added as surrogate standards to each sample prior to monitoring the analytical recovery efficiency before extraction, and the concentrations of all types of samples were corrected based on recoveries. The respective recoveries of $^{13}C_3$-CAF, $^{13}C_6$-SMX, D_5-NOX, and ^{13}C, D_3-ETM were 80–92%, 82–106%, 62–87% and 72–90% in water samples, 67–92%, 70–90%, 60–81% and 65–82% in sediment samples and 65–89%, 67–85%, 70–93% and 63–80% in biological samples, respectively. Instrumental detection limits (IDLs) were defined as 3 times the signal-to-noise (S/N) ratio, and

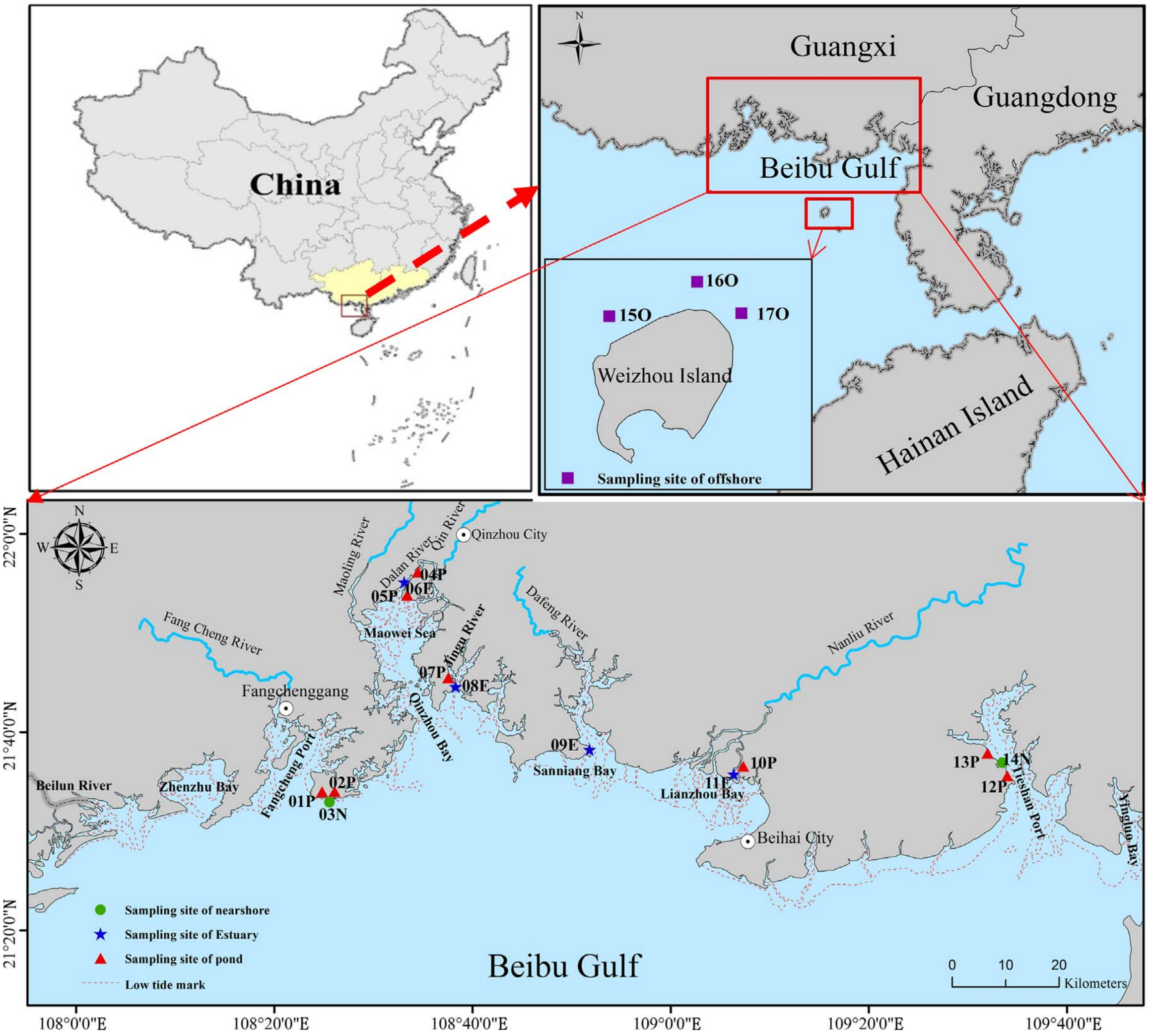

Fig. 1. Map of the sampling locations in the Beibu Gulf.

instrumental quantification limits (IQLs) were defined as 10 times the S/N (Zheng et al., 2012). The method detection limits (MDLs) of each antibiotic in water, sediment samples and biological samples ranged from 0.08 to 1.56 ng L^{-1}, from 0.004 to 0.35 ng g^{-1} and from 0.005 to 0.63 ng g^{-1} (Table S6), respectively.

2.5. Calculation of the bioconcentration factor (BCF)

The bioconcentration factor (BCF), which is used to evaluate the bioaccumulation of antibiotics in biota samples, was calculated according to the following equation:

$$BCF = \frac{Cbiota}{Cw} \times 1000$$

where the unit of the BCF is expressed as L kg^{-1}, C_{biota} is the concentration of antibiotics in biota samples (ng g^{-1}), and C_w is the contaminant concentration of antibiotics in water (ng L^{-1}) (Kinney et al., 2008). Chemicals are defined as "bioaccumulative" if they have a BCF greater than 5000 L kg^{-1} and as "potentially bioaccumulative" if they have a BCF ranging from 2000 to 5000 L kg^{-1} in biota samples (Wu et al., 2010).

2.6. Calculation of estimated daily intake (EDI)

Consumption of aquatic products containing antibiotics may pose a risk to the health of consumers (Boonsaner and Hawker, 2013). To supply safety information for aquatic product consumption for public reference, the EDI (ng d^{-1} $person^{-1}$) of the selected antibiotics was calculated according to the following equation:

$$EDI = C_{biota} \times M_{biota}$$

where C_{biota} (ng/g) is the maximum concentration of the selected antibiotics in a biota sample (wet weight) assuming a "worst-case scenario" (Hartmann et al., 1998) and M_{biota} (g d^{-1} $person^{-1}$) is the daily consumption amounts of selected seafood (shrimps, crabs or oysters) for a specific age group. The daily seafood consumption amounts in the study were obtained from a questionnaire-based dietary survey in the coastal areas of South China (Guo et al., 2010).

3. Results and discussion

3.1. Occurrence and distribution of antibiotics in water samples

The concentration of antibiotics in the water samples from culture ponds, estuaries, nearshore and offshore of the Beibu Gulf are summarised in Fig. 2 and Table S7. A total of 18 of the 19 targeted antibiotics were detected, with total concentrations ranging from 1.79 to 885 ng L^{-1} in the water samples. Of these 18 antibiotics, NOX, CIX, CTM, RTM, DETM and FF showed the highest detection frequencies of 100% in culture ponds, estuaries and nearshore. This indicates that these antibiotics are extensively used in the Beibu Gulf.

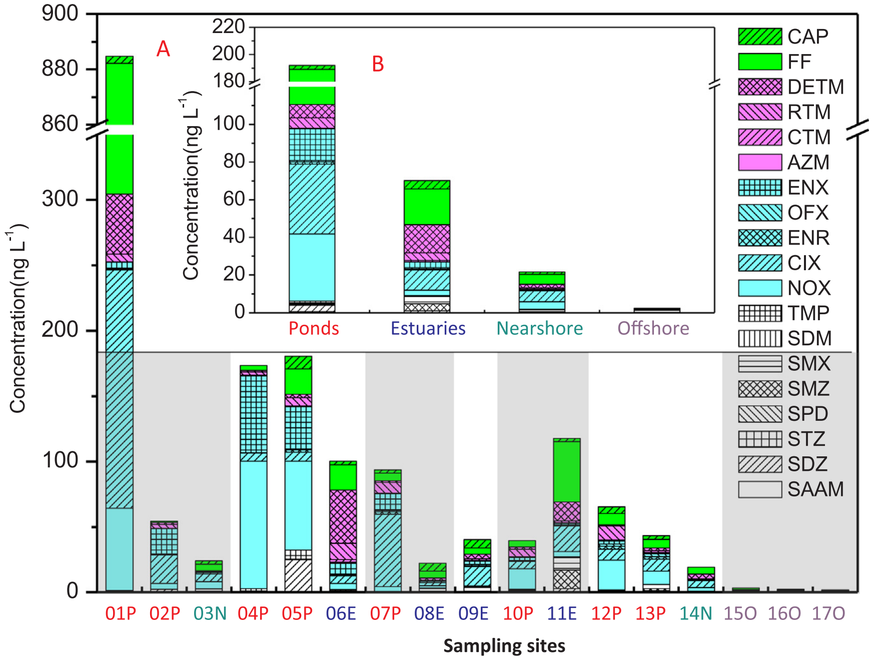

Fig. 2. Composition profiles of antibiotics in aquaculture water and natural seawater samples. (A: The concentration of antibiotics in each sampling site in the Beibu Gulf. B: The average concentration distribution of antibiotics in ponds, estuaries, nearshore and offshore sites of the Beibu Gulf. The sites are marked with different colours. Red represents culture ponds, blue represents estuaries, green represents nearshore, and purple represents offshore.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.1.1. *Culture ponds*

Seventeen of the 19 targeted antibiotics were detected in eight water samples from aquaculture ponds, with concentrations ranging from < MDL (CTM) to 578 ng L^{-1} (FF) (Fig. 2 and Table S7). The total concentrations ranged from 39.6 to 885 ng L^{-1} (mean: 192 ± 285 ng L^{-1}) in the aquaculture pond water, which were higher than in the estuary/open culture farms (range: 22.4–118 ng L^{-1}, mean: 70.2 ± 46.0 ng L^{-1}), nearshore seawater (range: 22.7–24.5 ng L^{-1}, mean: 23.6 ± 1.3 ng L^{-1}) and offshore (range: 1.79–3.23 ng L^{-1}, mean: 2.42 ± 0.74 ng L^{-1}). This demonstrates that aquaculture is an important source of antibiotic pollution in the coast of the Beibu Gulf. Among these antibiotics, seven antibiotics, including NOX, CIX, ENX, CTM, RTM, DETM and FF, were detected in all pond waters, indicating that these antibiotics occur widely in the aquaculture farms. Some banned veterinary antibiotics (NOX, CTM and RTM) (Ministry of Agriculture the PRC, 2005, 2015) remained on the above list, and the concentration reached 100 ng L^{-1} (NOX). FQs and CAPs were the predominant compounds, which accounted for 28.4–94.0% (mean: 47.7%) and 2.2–65.6% (42.4%), respectively, of the total concentration in the seven ponds. Of the two groups, NOX, CIX, ENX and FF showed a higher average concentration (16.9–78.5 ng L^{-1}) than the other antibiotics (0.77–2.83 ng L^{-1}). SAs and MLs, which accounted for a low proportion of the total concentration, presented low level concentrations. Most (97%) were lower than 10 ng L^{-1}, and 87% were lower than 1.0 ng L^{-1}. Compared to other studies, the antibiotic concentrations in the mariculture pond waters of the Beibu Gulf were generally higher than that in the mariculture ponds of Hailing Island, South China (Xu et al., 2006). NOR in the ponds of the Beibu Gulf was at a similar level to the aquaculture environment of the Pearl River Estuary, whereas OFL was lower but ENR was higher than that in the Pearl River Estuary (Liang et al., 2013).

Large variations of antibiotic concentrations occurred in the eight different culture ponds. The highest total antibiotic concentration (885 ng L^{-1}) occurred in the 01 P (adult shrimp pond) and was 20 times higher than the lowest concentration (39.6 ng L^{-1}) in the 10 P (shrimp larvae pond). The variable coefficient of the total concentration was 149%. The large variation may be caused by the different use of antibiotics and feed when the aquatic organisms are at different growth stages or health conditions. For example, 02 P cultured adult shrimp (individual weight range: 24.2–29.4 g) discharged most of the culture water into the surrounding sea and harvested the shrimp three days prior. A relatively lower antibiotic concentration was detected in this pond (Σantibiotics: 54.6 ng L^{-1}) than most of the other ponds (01 P, 04 P (shrimp larvae pond), 05 P (young shrimp pond) and 07 P (adult shrimp)). This phenomenon may be because the use of antibiotics was reduced before the aquatic products were harvested to reduce their residues and increase their food safety. The 01 P pond, which was close to the 02 P pond and cultured adult shrimp (individual weight range: 36.5–40.3 g), had the highest total antibiotic concentration (885 ng L^{-1}), particularly FF, CIX and DETM at significantly high concentrations of 578, 182 and 45.8 ng L^{-1}, respectively. This was because these drugs were applied in this pond a few days prior in order to treat bacterial diseases. The other ponds cultured shrimp at different growth stages (individual weight range: 3.2–15.0 g) and presented different antibiotic concentrations. However, no difference occurred between antibiotic concentrations in the water and the shrimp weight. Detailed parameters should be studied following the shrimp culture process.

3.1.2. *Natural water*

Compared to the mariculture ponds, the total antibiotic concentrations in the natural water (estuary and nearshore) near the ponds were at relatively low levels, except the Nanliu River estuary (11E). The Nanliu River is the largest river in Guangxi Province. It flows through some important animal breeding areas. Therefore, high antibiotic concentrations (total: 118 ng L^{-1}) were detected in this river, particularly the veterinary drug FF (46.0 ng L^{-1}) and the human and veterinary drugs SMZ (14.0 ng L^{-1}), SMX (9.8 ng L^{-1}), and DETM (14.4 ng L^{-1}), compared with the other three estuaries and two nearshore sites. The original brackish water in the 10 P pond (salinity: 4.1‰) came from the mixed water of the Nanliu River water and the Lianzhou Bay seawater. Therefore, the antibiotic concentrations in the 10 P pond were lower than in 11E, mainly because of seawater dilution when the Nanliu River water was discharged into the Lianzhou Bay. In additional to the Nanliu River, high antibiotic concentrations (total: 100 ng L^{-1}) were also detected in the Dalan River, which flows through the central urban area of Qinzhou City and discharges into the Maowei Sea. The other two river estuaries (08E and 09E) and two nearshore sites (03 N and 14 N) were located in the open sea area and were affected by the exchange of seawater. Therefore, they presented low level concentrations (total: 22.4–40.3 ng L^{-1}). Most antibiotics in these areas were at similar levels to those detected five years ago in the same areas (Zheng et al., 2012) and those in the open culture areas of Hailing Island, South China Sea, but were lower than those measured in the Pearl River estuary in South China (Soren, 2003), the coast of Bohai Bay, Jiaozhou Bay in the Bohai sea (Zhang et al., 2012b; Zou et al., 2011) and Jiaozhou Bay (Zhang et al., 2013) in the Yellow Sea. Compared to the nearshore area, the antibiotics in the sea area around Weizhou Island 40 km away from the mainland (15 O, 16 O and 17 O) were one order of magnitude lower (total: 1.81–3.23 ng L^{-1}). This further indicates that mariculture ponds and river discharge are important sources of marine antibiotic pollution.

3.2. Occurrence and distribution of antibiotics in the sediments

Thirteen of the 19 antibiotics were detected in sediment samples ranging from 0.023 (CTM) to 52.5 ng g^{-1} (NOX) (Table S6). The total concentration ranged from 3.22 to 83.2 ng L^{-1} (mean: 28.3 ± 27.5 ng L^{-1}) in the mariculture ponds. They showed larger variation than in the natural sediment environment (estuary and nearshore) (total concentration: 16.0 ± 4.1 ng L^{-1}). The phenomenon was similar to that in the water environment. The total concentrations in all sediments were positively ($p = 0.000$) related to those in the water samples at the same sites (Fig. S1). Compared to other areas, antibiotics in this area were generally higher than those in the mariculture farms at Hailing Island, South China Sea (Xu et al., 2006), similar to those in the Pearl River estuary (Liang et al., 2013). Of all antibiotics in the sediment samples, FQs were the most abundant antibiotics, followed by SAs. The two groups of compounds accounted for 91% − 100% of the total concentrations in all samples. Antibiotics in the two groups, such as NOX and SMX, were up to tens of ng L^{-1}. The other two groups of antibiotics, MLs and CAPs, were detected at low levels. Almost all were lower than 1 ng g^{-1}, with the exception of AZM in site 09E (1.34 ng g^{-1}). A previous study showed that these antibiotics can resist degradation in the marine sediment, particularly in quiescent systems (Xu et al., 2009). However, a limited number of studies was conducted on the persistence of antibiotics in mariculture sediment; therefore, their potential risks to the sediment environment remain to be evaluated (Fig. 3).

3.3. Occurrence of antibiotics in feed samples

In this study, 10 antibiotics were detected in three feed samples from 04 P (one feed for adult shrimp (04P-A), one feed for young shrimp (04P-Y)) and another feed for young shrimp (07P-Y), with concentrations ranging from 0.03 (CTM) to 95.4 ng g^{-1} (TMP) (Fig. 4-A and Table S10). The total concentration of the detected antibiotics in the adult shrimp feed 04P-A (105 ng g^{-1}) was higher than in the two young shrimp feeds (18.4 and 14.0 ng g^{-1}). In the adult shrimp feed 04P-A, TMP composed 91% of the total content with the highest concentration of 95.4 ng g^{-1}, followed by AZM and ENX with concentrations of 7.1 and 2.0 ng g^{-1}, respectively. In the young shrimp feed 04P-Y, three FQs (ENR, ENX and CIX) and one SA (SMZ) represented 94% of the total content, with concentrations ranging from 1.5 to 6.4 ng g^{-1}. In the young shrimp feed 07P-Y, SMX composed 81% of the total contents with the highest concentration of 11.4 ng g^{-1}, followed by ENX with a concentration of 2.2 ng g^{-1}. Except for the antibiotics described above, the other antibiotics all presented concentrations below 1 ng g^{-1}, and even below 0.1 ng g^{-1}. The results indicate that some prohibited antibiotics (Laabs et al., 2002; Ministry of Agriculture the PRC, 2001, 2015) were detected in feed in the study areas. Moreover, their contents showed large variations. The highest was nearly 10^4 times higher than the lowest. Therefore, it is estimated that those antibiotics with high contents may be added directly as feed additive and that those antibiotics with low contents may be residues in feed raw ingredients, such as trash fish or other components (Conti et al., 2015).

Compared to antibiotics in the feed samples, some antibiotics with high concentrations in the water (such as NOX in 04 P, CIX in 07 P), sediment (such as NOX in 04 P and 07 P) or biota (such as NOX and CIX in 04 P) samples were not detected in the feed samples or were detected at very low concentrations. Therefore, this suggests that shrimp feed is not the primary source of antibiotic residues found in water, sediment or shrimp muscle tissue. Another important observation was that antibiotics were mixed into farms' own prepared medicated feed when essential (Kim and Carlson, 2007; Luo et al., 2008).

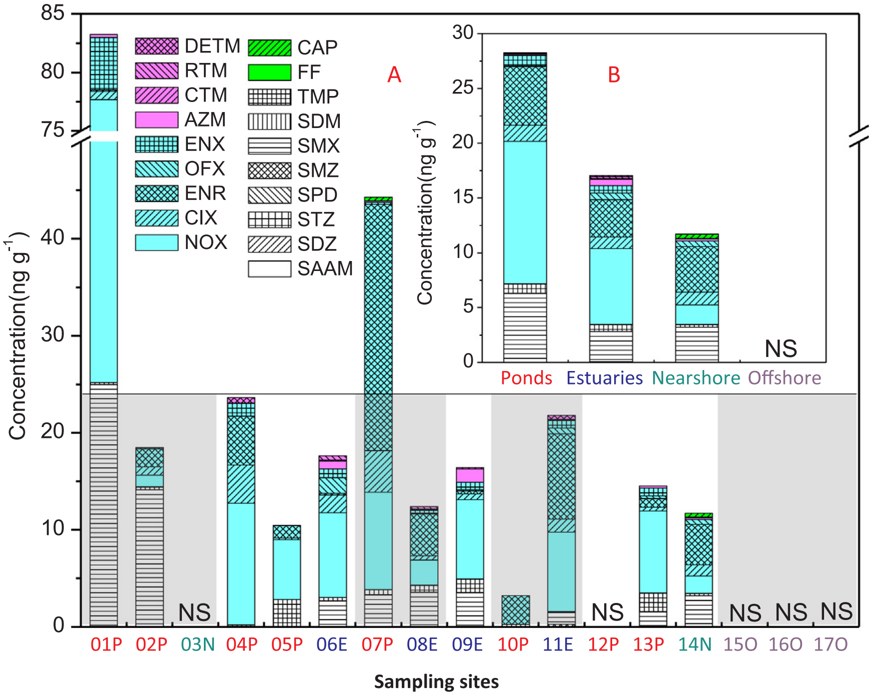

Fig. 3. Antibiotics in the sediments of aquaculture ponds and the natural environment in the Beibu Gulf. "NS" in the figure means no sediment for analysis.

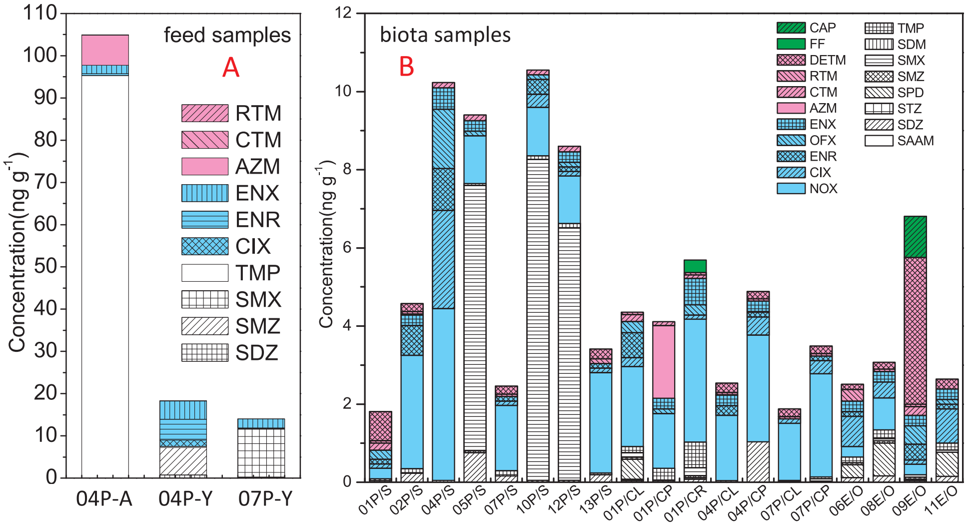

Fig. 4. Composition profiles of antibiotics in biota and feed samples from mariculture farms in the Beibu Gulf. S: shrimp, CL: crab leg muscle, CR: crab roe, CP: crab pereion muscle, O: oyster.

3.4. Occurrence of antibiotics in the biota samples

Twelve of the 19 antibiotics were detected in five shrimp samples with total concentrations ranging from 1.80 (01P-shrimp) to 10.6 ng g^{-1} ww (10P-shrimp) and individual antibiotic concentrations ranging from not detected to 8.21 ng g^{-1} ww (SMX) (Table S9 and Fig. 4-B). Total antibiotic concentrations in the shrimp showed a negative correlation with their growth stage (individual gross weight) (p = 0.000) (Fig. S2 in Supplementary information). Antibiotics in young shrimp with a low gross weight (3.7–14.8 g) reached higher total concentrations (8.59–10.6 ng g^{-1} ww) than in middle-aged and adult shrimps (2.65–4.67 ng g^{-1} ww). Especially in the adult shrimp from 01 P with a high gross weight (39 g), the total antibiotics concentration is only about 1.80 ng g^{-1} ww, but the concentrations of antibiotics in the water and sediment from 01 P were both the highest among all shrimp ponds. This may be due to the reducing use of antibiotics before the shrimp were harvested in order to pass stringent inspection of antibiotic residues. Notably, the relationship between antibiotic concentration in shrimp muscle and their growth stage should be further studied because samples collected in this study are likely not sufficient and dynamic changes in antibiotic concentrations in shrimp muscle with shrimp growth were not carried out.

Among the SA group, SDZ, SMZ, SMX and TMP were detected at frequencies of more than 60%, whereas SAAM and SDM were not detected in the biota samples. The SA antibiotic concentrations were higher than FQs, MLs and CAPs. The average concentration of SMX in shrimp was 2.68 ng g^{-1} ww, which was higher than that detected in oyster (< MQL) and crab (nd~0.20 ng g^{-1} ww). It was also higher than that detected in shrimp, crab and molluscs (not detected) in Hailing Island (Chen et al., 2015b), crab and shrimp samples in Baiyangdian Lake (Li et al., 2012), and organisms (nd~2.27 ng g^{-1} ww) in the coast of Dalian, Northeast China (Na et al., 2013). Five targeted FQs were found in shrimp at detection frequencies of more than 50% and total concentrations of up to 10.0 ng g^{-1} ww. Of these antibiotics, NOX showed the highest detection frequencies of 100%, with a maximum concentration of 4.40 ng g^{-1} ww. The FQ concentrations in this study were lower than that detected in the Asian shore crab (286.6 ng g^{-1} ww) in the Liao River Basin (Bai et al., 2014) and crab (24.2 ng g^{-1} ww) and shrimp samples (167 ng g^{-1} ww) in Baiyangdian Lake (Li et al., 2012). For MLs and CAPs, CTM, RTM and DETM were detected at frequencies of more than 75%, with concentrations ranging from nd~0.74 ng g^{-1} ww, whereas AZM, FF and CAP were below the MDLs in all shrimp. This shows that residual SAs and FQs are the main antibiotic contamination in shrimp.

The concentration of SMX in 10 P (8.21 ng g^{-1} ww), 05 P (6.79 ng g^{-1} ww) and 12 P (6.46 ng g^{-1} ww) was the highest of the 12 antibiotics detected in shrimp, whereas SMX was detected at a concentration close to or below the MDL in the water, and it was not detected in the sediments at the three sampling sites. In addition, SMX was detected in both the surrounding water and sediments, particularly in the Nanjiu River estuary (11E), which is located near 10 P, and where it reached at 9.8 ng L^{-1}. The reasons for this phenomenon may be because semi-closed breeding is susceptible to the influence of the surrounding seawater and SMX accumulate more easily in shrimp muscle.

Fifteen targeted antibiotics were detected in four oyster samples collected in the estuary of Dalan River (06E), Jingu River (08E), Dafeng River (09E) and Nanliu River, with total concentrations of up to 6.29 ng g^{-1} ww. Of these 15 antibiotics, four antibiotics, including SDZ, CIX, ENX and DETM, showed the highest detection frequencies of 100%, with a maximum concentration of 3.76 ng g^{-1} ww (DETM). However, the concentrations of other antibiotics were all below 1.0 ng g^{-1} ww. All detected antibiotics in oysters were not detected in the same oysters from Hailing Island, South China Sea (Chen et al., 2015b). However, one antibiotic, salinomycin, detected in oysters from Hailing Island was not analysed in this study.

For the three crab samples, 16 antibiotics were detected in different parts (two or three parts), with a total concentration ranging from 1.96 to 5.67 ng g^{-1} ww, which was similar to crabs (*Calappa philargius*) (nd-9.2 ng g^{-1} ww) from Hailing Island but lower than in the swimming crab (540 ± 60 ng g^{-1} ww) and Japanese stone crab (340 ± 30) collected in the natural sea area of Laizhou Bay, North China (Ikem et al., 2003). Of the different parts of crabs, NOX was the predominant antibiotics with the highest concentration ranging from 1.40 to 3.14 ng g^{-1} ww, which accounted for 45–74.7% of the total concentration. Of crab in 01 P, a higher concentration was detected in crab roe (5.67 ng g^{-1} ww) than that in leg muscle (4.34 ng g^{-1} ww) and pereion muscle (4.10 ng g^{-1} ww). A higher lipid content in the crab roe (47.3%) than in the other two parts (23.7% and 25.2%) (Table S3) and the organ properties may both cause higher antibiotic concentrations in the crab roe. First, high contents of lipids easily accumulate some antibiotics with a high K_{ow} value. Second, crab roe is composed of ovary and hepatopancreas. Hepatopancreas is an important digestive tissue, which may have higher bioaccumulation ability for contaminants. Similar results were present in digestive tissues of the wild fish collected in the East River, South China (Hartmann et al., 1998).

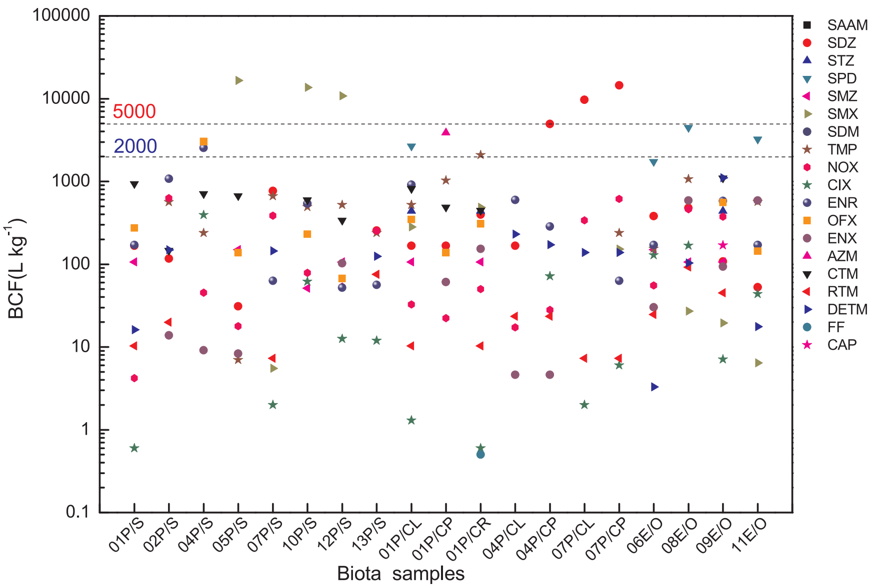

Fig. 5. Summary of the bioconcentration factor (BCFs) values in the study areas. (P: pond; E: estuary; S: shrimp; CL: crab leg muscle; CP: crab pereion muscle; CR: crab roe; O: oyster).

Table 1
EDI (ng d^{-1}) of antibiotics via seafood consumption and the relative contribution (%) of each seafood group to the total EDIs of SAs, FQs, MLs and CAPs.

EDI (ng d^{-1})	Children (2–5 years)		Youth (6–18 years)		Adult (> 18 years)	
	Male	Female	Male	Female	Male	Female
Sum of SAs						
Shrimp	28.4 (60.2)	17.3(60.6)	91.6(60.3)	56.8(60.3)	56.8(60.3)	54.1(60.6)
Crab	2.0 (4.2)	1.2(4.2)	6.3(4.1)	3.9(4.1)	3.9(4.1)	3.5(3.9)
Oyster	16.8 (35.6)	10.1(35.2)	54.1(35.6)	33.5(35.6)	33.5(35.6)	31.7(35.5)
Total	47.2	28.7[a]	152	94.2	94.2	89.3
Sum of FQs						
Shrimp	31.1(42.6)	19.1(42.1)	100.4(42.7)	62.2(42.8)	62.2(42.8)	59.2(43.1)
Crab	6.8(9.3)	4.2(9.3)	21.6(9.2)	13.2(9.1)	13.2(9.1)	12.1(8.8)
Oyster	35.1(48.1)	22.1(48.7)	113(48.1)	69.9(48.1)	69.9(48.1)	66.2(48.1)
Total	73	45.4	235	145.3	145.3	137.5
Sum of MLs						
Shrimp	3.5(5.8)	2.3(6.2)	11.8(6.0)	7.3(6.0)	7.3(6.0)	7.0(6.1)
Crab	3.0(4.9)	1.9(5.1)	9.6(4.9)	5.8(4.58)	5.8(4.8)	5.4(4.7)
Oyster	54.3(89.3)	32.7(88.6)	174.8(89.1)	108.1(89.2)	108.1(89.2)	102.4(89.2)
Total	60.8	36.9	196.2	121.2	121.2	114.8
Sum of CAPs						
Shrimp	1.3(11.2)	0.8(11.4)	4.2(11.2)	2.6(11.3)	2.6(11.3)	2.5(11.5)
Crab	1.08.6)	0.6(8.6)	3.1(8.3)	1.9(8.2)	1.9(8.2)	1.7(7.8)
Oyster	9.3(80.2)	5.6(80.0)	30.1(80.5)	18.6(80.5)	18.6(80.5)	17.6(80.7)
Total	11.6	7.0	37.4	23.1	23.1	21.8

Note:
[a] EDIs in blue and red represent the minimum and maximum values of total EDIs of the four class antibiotics, respectively.

3.5. Bioconcentration of antibiotics in biota samples

In this study, the BCFs of detected compounds ranged from 0.58 to > 16,555 L kg^{-1} in shrimp, from 0.55 to > 14,452 L kg^{-1} in crab and from 3.29 to 4482 L kg^{-1} in oyster (Fig. 5 and Table S11). SMX in the shrimp showed the highest BCF value with an average value of 5126 L kg^{-1} (range: 0- > 16,555 L kg^{-1}) of all detected antibiotics. It was higher than in organisms in the coast of Dalian (mean: 350 L kg^{-1}) (Na et al., 2013), Hailing Island (median: 185 L kg^{-1}) (Chen et al., 2015b) and Liao River Basin (maximum: 65 L kg^{-1}) (Bai et al., 2014). It showed that SMX easily accumulated in shrimp. ENR and OFX were accumulated by shrimp in the 04 P pond with BCFs of > 2542 L kg^{-1} and > 3040 L kg^{-1}, whereas the other values were all below 1100 L kg^{-1} in the shrimps from other sites and the crab from the 04 P pond. In crab, the BCFs values of SDZ ranged from 9691 to 14,452 L kg^{-1} in the 07 P pond, which was higher than that in fish (*Trachinotus ovatus*) (781 L kg^{-1}) and shrimp (*Fenneropenaeus penicillatus*) (1392 L kg^{-1}) from the Hailing Island, South China Sea (Chen et al., 2015b), and similar to the average BCF of SDZ (10,757 L kg^{-1}) in *Crassostrea gigas*, *Patinopecten yessoensis,* and *Chlamys farreri* from the coast of Dalian, North China (Na et al., 2013). It indicated that SDZ was bioaccumulative in crab. Other compounds had BCFs values lower than 2000 L kg^{-1}, whereas the BCFs of SPD, TMP and AZM were > 2673 L kg^{-1}, 2094 L kg^{-1} and > 3874 L kg^{-1} in crab, respectively. SPD was accumulated by oyster with the average BCFs of 2363 L kg^{-1} (range: 0- > 4482 L kg^{-1}), while other antibiotics had BCFs values lower than 1100 L kg^{-1}. In addition, no MLs and CAPs accumulated in shrimp, crab and oyster, except AZM in the crab pereion muscle (01 P/CP) because all BCFs were below 1200 L kg^{-1}. These results suggest that SMX and SDZ are bioaccumulative, whereas ENR, OFX, SPD, TMP and AZM are potentially bioaccumulative. Therefore, the risks posed to aquatic organisms cannot be overlooked.

However, it should be noted that the BCF calculated above has some limitations. The antibiotic concentrations in the water and biota samples showed large variation at different growth stages because of feed supply or disease treatment. In addition, the geochemical behaviour of antibiotics, such as sorption, photo-degradation and microbial degradation, also affect their bioconcentration in aquatic organisms.

3.6. Estimated daily intakes (EDIs) of antibiotics via seafood consumption

The daily intake of antibiotics from the consumption of three seafood groups (i.e. shrimp, crabs, and oyster) was evaluated according to the method described in Section 2.6. The EDIs of SAs, FQs, MLs and CAPs in aquatic products for different age groups were 28.7–152, 45.4–235, 36.9–196.2 and 7.0–37.4 ng d^{-1}, respectively (Table 1). For SAs and FQs, the concentrations of shrimp and oyster to the EDI was above 90%. For MLs and CAPs, the contribution of oyster to the EDI was above 88% and 80%. Acceptable daily intakes (ADIs) and maximum residue limits (MRLs) were used to assess the health risk, as these antibiotics were banned by the Ministry of Agriculture of the People's Republic in China (Ministry of Agriculture the PRC, 2002), the Japan Food Chemical Research Foundation and some other organization (Table S13). The MRLs for these antibiotics were between 20 and 100 ng g^{-1}, and the concentrations of all selected antibiotics in shrimp, oysters and crabs were all less than the corresponding standard in this study. This indicates that these aquatic products have reached the standard for safe consumption. In addition, the EDI values of these targeted antibiotics were all below the ADIs, suggesting that they could not pose a health risk to humans. In the actual environment, there are many types of antibiotics in the muscle tissue of aquatic products, but the health risk assessment of this study does not consider the role of mixed antibiotics, which may have uncertain risks to human health.

4. Conclusion

This investigation studied the occurrence, bioconcentration and human dietary exposure of 19 antibiotics from different farming models (closed pond culture and open estuary culture), biota (shrimp, crabs and oysters), growth stages (young and adult), feed and environmental matrices (water and sediment) in typical mariculture farms, estuaries (open mariculture regions), and nearshore and offshore areas of the Beibu Gulf. Generally, higher concentrations of antibiotics were detected in culture ponds than in natural sea areas, clearly demonstrating the wide use of antibiotics in pond culture farms. The detection of 10 antibiotics with a wide range of concentrations in feed samples indicated the use of antibiotics in the feed as an additive and/or the residual of antibiotics in the raw material of the feed. The BCFs of the antibiotics suggest that SMX and SDZ is bioaccumulative, whereas ENR, OFX, SPD, TMP and AZM are potentially bioaccumulative. The risks posed to aquatic organisms cannot be overlooked. The seafood health risk assessment demonstrated that these aquatic products (shrimp, crab and oyster) have reached the standard for safe consumption and could not pose a health risk to humans. However, the presence of multiple antibiotics may pose a potential risk to humans, particularly for sensitive populations, such as pregnant women and children. Thus, the problem of antibiotic abuse should cause the attention of the relevant departments. Effective measures must be put forward to enhance seafood safety in China.

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Conflict of interest

All the authors have no competing interests to declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2018.02.006.

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