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**Research** article

## Investigation of the interaction between the fate of antibiotics in aquafarms and their level in the environment



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### ABSTRACT

China is the largest freshwater aquaculture producer and antibiotics consumer in the world, and rivers in China are generally polluted by antibiotics. However, there is little information available regarding the linkage of antibiotics in aquaculture and the aquatic environment. Therefore, this study investigated the fate of antibiotics in several open water culture-based freshwater aquafarms, including integrated livestock/fish systems and non-integrated fish ponds, and explored the contamination profiles of antibiotics in the Beijiang River. Then the study tried to clarify the two-way interaction of antibiotics in aquaculture and the environment. The results showed that, when compared with the effluent from livestock farms and wastewater treatment plants, the contribution of antibiotics from non-integrated fish pond water without livestock sewage input was limited, while that of effluent from the integrated livestock/fish system was quite high. The total concentrations of antibiotics detected in the aquafarm source water were similar to those in the upper river water and generally higher than those in the corresponding fish pond water, implying that the occurrence of antibiotics in intensive aquafarms can mainly be attributed to the antibiotic residues in nearby river water. Overall, the results underscore the need to develop a sewage infrastructure for the treatment of effluent from integrated livestock/fish aquafarms, and suggest that open water culture-based fish farms should be located far from seriously contaminated sections of rivers.

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#### 1. Introduction

Antibiotics are extensively used to prevent or treat bacterial infections in humans, livestock and aquaculture. The annual usage of antibiotics in China is approximately 162,000 tons (Zhang et al., 2015), which is almost one order of magnitude higher than that in the United States (Kummerer, 2009). After application, these antibiotics are transported to municipal (Tran et al., 2016), livestock (Zhou et al., 2013a) or aquaculture wastewater (Chen et al., 2015a). Because of the limited wastewater infrastructure and incomplete removal in wastewater facilities (Zhou et al., 2013b), the residual antibiotics are finally directly or indirectly discharged into the receiving environment, where they can cause toxic effects on aquatic organisms (Yang et al., 2008; Zounkova et al., 2011) and aggravate the development of bacterial antibiotic-resistance in the aquatic environment (Luo et al., 2010; Zhang et al., 2014). When compared with wastewater treatment plants (WWTPs) (Zhou et al., 2013b; Tran et al., 2016) and livestock farms (Zhou et al., 2013a, 2013c), few studies have investigated the fate of antibiotics in different water samples (e.g., source water and fish pond water) of freshwater aquafarms with different aquaculture species (e.g., grass carp, mandarin fish and mud carp) and aquaculture modes (e.g., non-integrated pond and integrated livestock/fish farms). Based on announcements from the Ministry of Agriculture of the People's Republic of China (No. 1997 and No. 2069) (MAOPRC, 2013, 2014),

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the Agricultural Industrialization Standard of the People's Republic of China (NY 5071-2002) (MAOPRC, 2002a), and the Aquacultural Industrialization Standard of the People's Republic of China (SC/T 1083-2007, SC/T 1084-2006 and SC/T 1085-2006) (MAOPRC, 2006a, b, 2007), certain antibiotics (*e.g.*, sulfamethazine, sulfamethoxazole, enrofloxacin, florfenicol, lincomycin and trimethoprim) have been approved for application in aquaculture. Since the majority of aquafarm wastewater is released directly into surrounding rivers, antibiotics may constantly reach the aquatic environment. In addition, China is the largest aquaculture producer in the world, accounting for two-thirds of the global aquaculture production (FAOUN, 2016). Thus, the transportation of antibiotics from aquafarms to the environment should be of concern.

Open water culture-based fisheries are the primary aquaculture mode in many Asian countries, including China and Vietnam (Edwards, 2015). In open water culture-based fisheries, fish pond water originates from open water bodies such as surface water or reservoir water. Waste and natural food in open water bodies is introduced as nutrition for farmed aquatic organisms. However, pollutants also enter fish ponds (Fuhrimann et al., 2016), leading to an uncertain influence on aquatic products. Many studies have reported that antibiotics are ubiquitous in river water from different investigated zones (Zheng et al., 2012; Zhang et al., 2015; Zhou et al., 2016). They were also found in pond water and cultured fish muscle samples of freshwater/marine aquaculture farms (Chen et al., 2015a; He et al., 2016). Moreover, antibiotics accumulated in cultured aquatic products could pose a potential risk to human safety based on daily intake estimation (Chen et al., 2015a). Accordingly, it is essential to understand the impact of antibiotics from the environment on aquafarms to ensure the quality and safety of products produced in such facilities.

The Beijiang River is an important drinking water source in South China that flows through the cities of Shaoguan, Qingyuan and Foshan, which contain many agriculture, aquaculture and industrial facilities. Although Ling et al. (2013) reported the occurrence and distribution of antibiotic resistance genes in the Beijiang River, they provided little information about the pollution level of antibiotics. Therefore, the Beijiang River was selected to assess the relationship of antibiotics in aquaculture facilities and the environment.

This study was conducted to investigate the fate of antibiotics in various types of aquafarms, to clarify the contribution of antibiotics from aquafarms to the environment in comparison with other pollution sources such as WWTPs and poultry farms, to explore the contamination profiles of antibiotics in the Beijiang River, and to understand the linkage of antibiotics in aquaculture-environment systems. The results presented herein provide important insight that will enable a better understanding of the two-way interaction of antibiotics in aquaculture systems and the environment. Based on the results of this study, countermeasures to alleviate the environmental pollution caused by antibiotics from aquaculture wastewater and to ensure the quality and safety of aquatic products from open water culture-based fisheries are proposed.

#### 2. Materials and methods

#### 2.1. Sampling sites and sample collection

The investigated area and sampling sites are illustrated in Fig. 1. The cities of Shaoguan, Qingyuan and Foshan with the population of 2.93, 3.83 and 7.43 million are located along the Beijiang River (GSY, 2016). Due to the developed agriculture and prosperous aquaculture, the Beijiang River and its surrounding aquafarms were selected to investigate the interaction of antibiotics between aquaculture facilities and the aquatic environment. There is also an important steel plant in Shaoguan, indicating heavy industrial activities in the investigated zone. In addition, the final effluents from two wastewater treatment plants located in this area were sampled. Detailed information regarding the sampling sites is presented in Tables S1–S3.

Sampling campaigns were conducted in August of 2015. One liter of water was collected in clean amber bottles, after which methanol (5% v/v) and 4 M H<sub>2</sub>SO<sub>4</sub> were added immediately to prevent microbial growth and adjust the pH to 3. The surface water and WWTP effluent were flowing, but the water in aquafarms was stagnant, indicating the concentrations of antibiotics in aquafarm water were more stable than those in surface water and WWTP effluent. Thus, for the surface water and WWTP effluent samples, there were three replicates of each sampling site, while there were no replicates of the water samples from freshwater aquafarms. Actually, it would have been better to get three replicates for aquafarm water samples. All collected water samples were consecutively placed in coolers, transported to the laboratory and then stored at 4 °C in a refrigerator before further processing, which was conducted within 24 h. Common aquatic products in southern China (e.g., grass carp, crucian carp, mud carp, mandarin fish, Nile tilapia and white leg shrimp) were collected by fish netting from six freshwater aquafarms (A2-A7; Table S4). The captured fish were promptly anaesthetized and sacrificed by dissection on the spot. After elimination of the skin, fish muscle was cut into small pieces, placed in polyethylene bags within a warm-box, brought to the laboratory and stored at -20 °C until extraction.

#### 2.2. Sample extraction and instrumental analysis

Twenty-seven antibiotics were investigated in this study, sulfadiazine (SDZ), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfamonomethoxine (SMM), sulfaquinoxaline (SQX), sulfachlorpyridazine (SCP), sulfapyridine (SPD), sulfamerazine (SMR), sulfameter (SM), sulfathiazole (STZ), sulfadimethoxine (SDM), anhydro-erythromycin (ETM-H<sub>2</sub>O), roxithromycin (RTM), clarithromycin (CTM), tylosin (TLS), enrofloxacin (EFX), norfloxacin (NFX), ciprofloxacin (CFX), ofloxacin (OFX), fleroxacin (FLX), oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC), florfenicol (FF), chloramphenicol (CAP), lincomycin (LIN) and trimethoprim (TMP). Their detailed information and abbreviations are provided in Tables S5–S6. Supplier sources of the chemicals and reagents can be found in the Supplementary material (Text S1).

Antibiotics in water samples were extracted using solid phase extraction as previously described by Zhou et al. (2012), while these compounds in fish muscle samples were extracted by the quick, easy, cheap, effective, rugged and safe method (QuEChERS) (Anastassiades et al., 2003). Briefly, water samples were filtered through 0.7 µm glass fiber filters (Whatman GF/F) before being spiked with internal standards. The filtered water samples were then extracted using Oasis HLB cartridges (500 mg, 6 mL), which were subsequently dried and eluted with 12 mL of methanol. The final eluate was then evaporated under a gentle nitrogen stream, reconstituted in 1 mL of methanol, and kept at -20 °C until HPLC-MS/MS analysis. For fish muscle samples, approximately 2.0 g (wet weight, ww) of muscle was placed into a 50 mL polypropylene centrifuge tube. Internal standards were then spiked accurately, after which the tube was placed in a fume cupboard overnight. The tissue combined with 10 mL of 0.1% (v/v) formic acid in acetonitrile was homogenized using an ultra-turrax homogenizer (IKA, Germany) at 30,000 rpm. Next, a mixture of 100 mg primary secondary amine (PSA), 40 mg C<sub>18</sub> and 600 mg anhydrous MgSO<sub>4</sub> was added to the tube, vortexed for 30 s, extracted by ultrasonic treatment for 15 min and centrifuged at 4500 rpm for 5 min. It is well known that PSA is an effective clean-up sorbent for eliminating polar impurities Download English Version:

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