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Nutrients, heavy metals and microbial communities co-driven distribution of antibiotic resistance genes in adjacent environment of mariculture☆

Zelong Zhao [a], Jing Wang [a, *], Ying Han [a], Jingwen Chen [a], Guangfei Liu [a], Hong Lu [a], Bin Yan [b], Shiaoshing Chen [c]

[a] *Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education), School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China*
[b] *School of Environmental Science and Engineering, Xiamen University of Technology, Xiamen 361024, China*
[c] *Institute of Environmental Engineering and Management, National Taipei University of Technology, Taipei 106, Taiwan, ROC*

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ABSTRACT

With the rapid development of aquaculture, the large amounts of pollutants were discharged into the aquatic environment, where the detected antibiotic resistance genes (ARGs) have drawn increasing attention due to their potential threats to ecological environment and human health. Thus, the impact of mariculture on ARGs was assessed and the underlying mechanism of their propagation was explained. Sediments from eight sampling sites were collected along a mariculture drainage ditch, and the sediment in Yellow River Delta National Park was used as a non-mariculture control. Microbial ARGs qPCR array and illumina sequencing of 16S rRNA gene were applied to examine the changing patterns of ARGs and bacterial communities. Results showed that 18 ARGs (3 fluoroquinolone, 1 aminoglycoside, 3 macrolide-lincosamide-streptogramin B, 2 tetracycline, and 9 beta-lactam resistance genes) were influenced by mariculture, and ARGs abundance and diversity were significantly increased in mariculture sediments ($p < 0.05$). A remarkable shift in bacterial community structure and composition was also observed. The abundance of most of ARGs were significantly decreased in the estuary samples, implying that seawater had a significant dilution effect on the ARGs emission from the mariculture sites. Partial redundancy analysis showed that nutrients, heavy metals, and bacteria communities might directly and indirectly contribute to ARGs propagation, suggesting that the profile and dissemination of ARGs were driven by the combined effects of multiple factors in mariculture-impacted sites.

1. Introduction

Recently, growing attention has been paid to the worldwide emergence and spread of antibiotic resistance genes (ARGs) owing to their unfavorable consequences of human health in modern medicines (Levy and Marshall, 2004). The increase of antibiotic resistance in pathogens bequeathed by the ARGs can complicate the treatment of diseases (Cosgrove et al., 2004) and has resulted in more than two million infections and 14,000 deaths each year in the United States (WHO, 2001). It was reported that the expansion of aquaculture has accelerated the prophylactic and therapeutic usage of antimicrobials, among which some of them were crucial in clinical therapeutics (Cabello et al., 2013). In general, the large number of incomplete metabolites and intact antibiotics exist in aquaculture sites, which has promoted the development of antibiotic resistance (Liang et al., 2013; Sapkota et al., 2008). Aquaculture pond is considered as one kind of the reservoir of ARGs (Seyfried et al., 2010; Xiong et al., 2015). Diverse ARGs have been detected in water and sediment of aquaculture and effluent-receiving aquatic environments (Buschmann et al., 2012; Di Cesare et al., 2013; Harnisz et al., 2015; Nonaka et al., 2007).

The awareness of antibiotic misuse harmful to human health has stimulated the surveillance on antibiotic administration (Laganà et al., 2011; Scarano et al., 2014). Denmark has banned the use of antibiotics as growth promoters (Aarestrup et al., 2010). In the

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* Corresponding author.
E-mail address: jwang@dlut.edu.cn (J. Wang).

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United States, the antibiotics used for human medicine will also cease for animal growth promotion after 2016, and veterinary oversight will be required for therapeutic uses (White House, 2015). However, antibiotics are not the only selective pressure of ARGs in aquaculture areas. Recent studies showed that ARGs were detected even in the aquaculture environments where antibiotics were never used. For example, antibiotic-resistant bacteria had been isolated from the fish, feed and environmental samples in an aquaculture ecosystem with no known antibiotics history (Huang et al., 2015). Tetracycline, sulphonamide and trimethoprim resistance genes persisted in a fish farm of the Baltic Sea, in which antibiotics were stopped to use 6 years ago (Muziasari et al., 2014; Tamminen et al., 2011b). Several mechanisms affecting the dynamics of ARGs were found in different environments. Horizontal gene transfer (HGT) mediated by mobile genetic elements such as integrons and plasmids has been demonstrated as the major driving force in the dissemination of ARGs (Chen et al., 2013; Luo et al., 2014; Szczepanowski et al., 2008). Additionally, co-selection of antibiotic and heavy metal resistances resulted from heavy metals, particularly Cd, Hg, Cu, and Zn was frequently in water and soil (Seiler and Berendonk, 2012). Moreover, recent studies have proved that bacterial community variation has a more contribution to resistome than HGT in soil, sludge and drinking water (Forsberg et al., 2014; Jia et al., 2015; Su et al., 2015). Thus, more exhaustive mechanisms of ARGs dynamics in environments influenced by aquaculture should be revealed for reducing the risks of ARGs.

To date, most studies on ARGs in aquaculture environment focused on a few human pathogens, such as *Aeromonas* (Deng et al., 2014; Han et al., 2012), *Vibrio* (Aedo et al., 2014; Reboucas et al., 2011) and *Enterococci* (Di Cesare et al., 2013) by using culture-dependent methods. Some researches analyzed ARGs in aquaculture environment by culture-independent methods, however, most of them merely targeted at a few specific ARGs according to their research purposes (Harnisz et al., 2015; Muziasari et al., 2014). Until now, there is no research obtained the comprehensive ARGs profile and how aquaculture activities drove the distribution of ARGs in aquaculture environment. Furthermore, the bacterial community composition in aquaculture environment has not been entirely explored. The objectives of this study were to investigate the response of ARGs and bacterial community to mariculture activities and the factors which involved in the ARGs shift in mariculture sediments. To the best of our knowledge, this paper makes the first pioneer to investigate ARGs variation in aquaculture environment using the microbial qPCR array technique.

2. Materials and methods

2.1. Sample collection and DNA extraction

Surface sediment samples (~500 g) were collected at 8 stations along a mariculture drainage ditch (N 37°34′44.0″, E 118°56′24.9″) in June (wet season) and October (dry season) 2014 in Dongying, China (Fig. 1). Different seasons are of different natural conditions such as temperature, rainfall and salinity, which can change the physical and chemical properties and microbial community of the research sites (Patel et al., 2014; Suh et al., 2015; Kaevska et al., 2016). Shrimp and sea cucumber were reared in the mariculture ponds around the drainage ditch. In front of the mariculture facilities, there are some houses regularly connected to sewage systems which were not linked with the drainage ditch. The sediments on

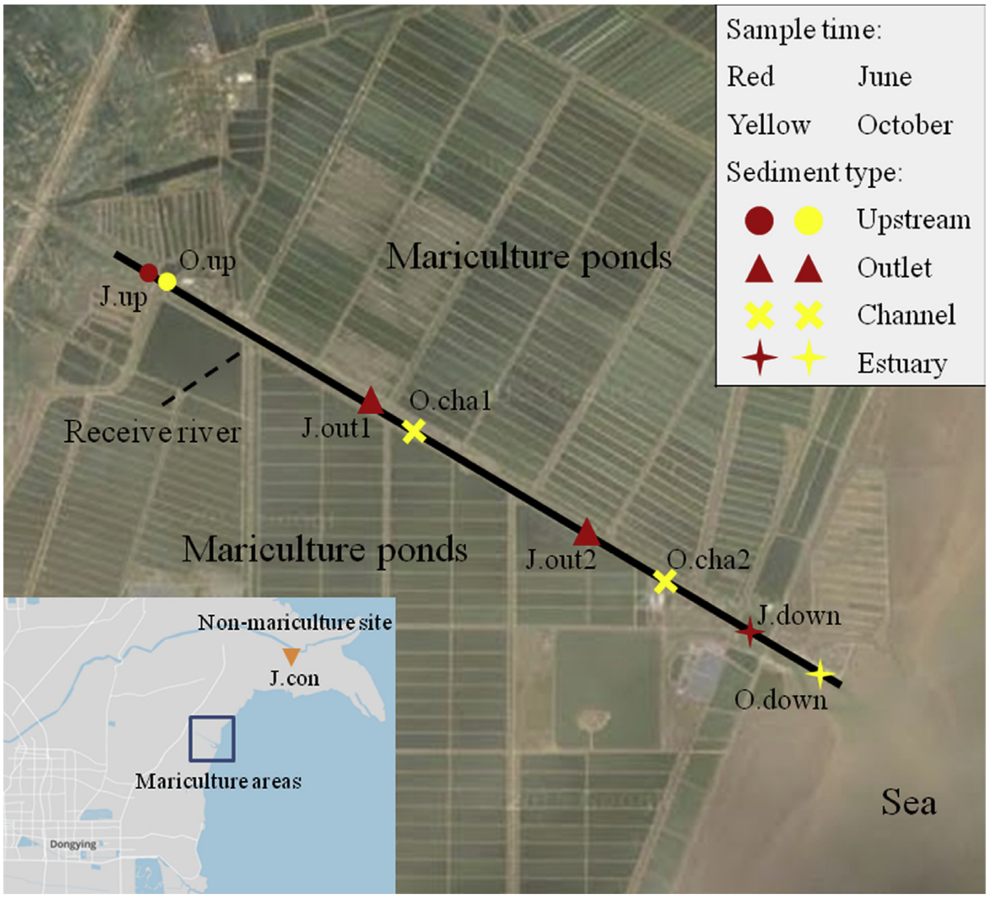

Fig. 1. Map of the sampling sites.

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