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Nutrient enrichment during shrimp cultivation alters bacterioplankton assemblies and destroys community stability

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ABSTRACT

Intensive shrimp farming is generally accompanied by nutrient enrichment and gradual eutrophication, which impose major threats to shrimp culture ecosystems. However, little is known about how the bacterioplankton community in a rearing environment responds to increased eutrophication during shrimp culture processes. In this study, we used the MiSeq sequencing technique to explore the impacts of nutrient enrichment on the assembly and stability of the bacterioplankton community. Our results showed that magnitudes of the changes in the bacterioplankton community compositions (BCCs) and diversity were closely associated with eutrophication level. Moreover, a phylogenetic-based mean nearest taxon distance (MNTD) analysis revealed that increased eutrophication significantly ($P < 0.01$) changed the bacterioplankton ecological processes from deterministic to stochastic. A structural equation model showed that eutrophication indicators affected the BCCs either directly by controlling resources or indirectly by modifying other environmental variables of the shrimp ponds in complex pathways. Furthermore, association network comparisons revealed that nutrient enrichment increased the complexity of interspecies interactions and the proportion of cooperative interactions and decreased the proportion of generalists, which suggest that nutrient enrichment destroyed the community stability. These findings suggest that minimizing nutrient pollution, especially at the end of cultivation, could be an important management tool for establishing a microbially mature water system.

1. Introduction

Intensive shrimp rearing spans many countries in Asia because of the high economic returns of shrimp farming (Pham et al., 2010). However, the management practices for shrimp farming may induce rapid nutrient enrichment in water ecosystems from inorganic nutrients and gradual eutrophication, thereby causing the deterioration of water quality, especially during the mid-to-late crop periods (Ma et al., 2013; Sugiura et al., 2006; Xiong et al., 2014). Disease outbreaks generally occur during particular phases of eutrophication in pond ecosystems (Lemonnier et al., 2010). The elevated nutrient conditions exert strong pressures on shrimp health by suppressing the immune system (Liao et al., 2012; Liu and Chen, 2004) and disturbing physiological processes (Furtado et al., 2016; Mugnier and Justou, 2004; Romano and Zeng, 2013). In addition, elevated nutrient conditions also cause resource variations in the water bodies associated with the culture (Ferreira et al., 2011). Given the bio-indicative significance of bacterioplankton that capture anthropogenic perturbations (Lemonnier et al., 2010; Ponsard et al., 2013; Xiong et al., 2016a), shifts in water resources may trigger changes in the surrounding bacterioplankton community (Dai et al., 2017b; Xiong et al., 2015; Xu et al., 2014), which, in turn, pose health risks to reared shrimp (Zhang et al., 2014). Several recent studies have consistently shown the responses of bacterioplankton communities in rearing environments to nutrient enrichment and their effects on shrimp health (Lemonnier et al., 2010; Lucas et al., 2010; Xu et al., 2010; Zhang et al., 2014). However, these studies mostly focused on specific taxa and particularly focused on potential shrimp pathogens (Liu and Chen, 2004; Lucas et al., 2010), which provide limited information at the community level. In fact, the high mortality of shrimp is attributed to the proliferation of opportunistic pathogenic bacteria rather than specific obligate pathogenic bacteria (Attramadal et al., 2014; Dai et al., 2017a; Xiong et al., 2017b). Opportunistic pathogenic bacteria are pervasive in sea water and will become dominant when the community composition changes and/or the community stability is interrupted (Skjermo et al., 1997). Therefore, it is more meaningful to study the response of bacterioplankton to nutrient enrichment at the community level.

An ultimate goal of microbial ecology projects is to predict the

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responses of bacterial communities to environmental forcing, yet this goal is difficult to achieve. One reason for this difficulty is that there are two types of processes - deterministic and stochastic - that influence the assembly of communities (van der Gast et al., 2008). Determinism refers to the processes of environmental filters and biotic interactions that determine the presence/absence and relative abundances of species, whereas stochastic processes assume that species exhibit probabilistic dispersal and random changes in relative abundances, which increase the unpredictability of a community assembly (Chase and Myers, 2011). It has been perceived by community ecologists that both deterministic and stochastic processes occur simultaneously during the assembly of local communities (Chase, 2010; Chase and Myers, 2011; Kim et al., 2013; Ofiteru et al., 2010; Zhou et al., 2014); however, no consensus has emerged regarding the relative importance of these processes in a habitat impacted by eutrophication. For example, consistent shifts from stochastic to deterministic-based processes are detected with increasing eutrophication (Dai et al., 2017b; van der Gast et al., 2008). In contrast, Zhou et al. (2014) reported that nutrient input was believed to increase compositional stochasticity by enhancing ecological drift and weakening niche selection. These divergent conclusions raise the question of whether and how the relative importance of stochastic vs. deterministic processes varies across disparate nutrient conditions during shrimp cultivation.

Another reason it has been so difficult to establish clear links between bacterioplankton community composition (BCC) and environmental forcing is that this link is not direct, but rather may be mediated by the interspecies interactions inherent in a community (Dang and Lovell, 2016; Little et al., 2008; Shade et al., 2012). In a given community, every member frequently interacts with other members to form a complex ecological network through various types of interactions, which could be either positive (e.g., mutualism and symbiosis) or negative (e.g., competition and parasitism) (Faust and Raes, 2012; Yang et al., 2018). Such networks are rich in structural heterogeneity (Olesen et al., 2007). Understanding a network structure and its underlying causes are essential parts of any study of community composition and its responses to perturbations. In addition, ample studies have indicated that topological properties of such networks affect the relationship between network complexity and stability (Deng et al., 2016; Shade et al., 2012; Xiong et al., 2017a; Zhu et al., 2016). For example, compositional diversity and connectivity lead to higher network complexity, thereby destabilizing community stability (Thebault and Fontaine, 2010). These findings led us to explore the extent of bacterioplankton community stability that is affected by nutrient enrichment during shrimp cultivation.

Shrimp farming is a nutrient enrichment process, but its effect on the bacterioplankton community in the rearing environment is poorly understood. In this study, we collected water samples from six shrimp (*Litopenaeus vannamei*) culture ponds to explore the effects of nutrient enrichment on the assembly and stability of the bacterioplankton community. To achieve this, we integrated structural equation modelling (SEM) (Hershberger, 2001), standardized effect size measures (Stegen et al., 2013), and network inference approaches (Deng et al., 2012) to address the following questions: (i) whether and how does nutrient enrichment affect the ecological processes of a bacterioplankton community? (ii) Is nutrient enrichment the decisive factor in determining the changes in the BCCs in shrimp culture ponds? (iii) What is the relationship between the bacterioplankton community stability and eutrophication level? The understanding of the ecological processes of a bacterioplankton community that are altered by nutrient enrichment in shrimp culture ponds can help us establish a microbially mature water system and solve some of the disease issues faced by shrimp farmers.

2. Materials and methods

2.1. Experimental site

The intensive rearing ponds investigated in this study were built in Zhanqi, Ningbo, eastern China (29°32′N, 121°31′E). These ponds were approximately the same size (2000 m^2) and were identically managed in terms of sea water input, daily water exchange rate (5%), depth (1.5 m), shrimp stocking density (360,000 ind. $Pond^{-1}$), feed type and schedule. The ponds were located in greenhouses to maintain a relatively stable temperature during the cool season. Bottom aeration was applied to maintain a suitable level of dissolved oxygen. Shrimp (L. *vannamei*) juveniles were introduced to the ponds on 8 April 2016 and harvested on 10 July 2016.

2.2. Water sample collection and analysis

Samples were taken at various time points separated by 6–10 days (over a span of 87 days from 15 April to 10 July) in six selected ponds. Water temperature (WT), pH, salinity (SAL) and dissolved oxygen (DO) were recorded in situ with a YSI 6000 multiparameter probe (YSI Inc., Yellow Springs, USA) at a depth of 50 cm. To reduce the spatial variability within the ponds, all water samples were mixed with water taken with an integrator tube from four representative points (similar locations in all ponds). All water samples were stored in the dark at 4 °C and were returned to the laboratory for further processing.

The levels of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) were analysed following standard methods (AQSIQ, 2007). For the analyses of ammonia (NH_4^+), nitrite (NO_3^-), nitrate (NO_2^-), and orthophosphate (PO_4^{3-}), samples were filtered through glass fibre filters (GF/F, 25 mm, 0.7 μm) with a filtration system (Vacuum Pump XF5423050, Millipore, Darmstadt, Germany) and measured with an automated spectrophotometer (Smart-Chem 200 Discrete Analyzer, Westco Scientific Instruments, Brookfield, USA). The eutrophication index (EI) was calculated as: $EI = DIN \times DIP \times COD \times 106/4500$ (AQSIQ, 2007), where DIN is the dissolved inorganic nitrogen content (the sum of ammonia, nitrate and nitrite levels, expressed in $mg\,L^{-1}$), DIP is the dissolved inorganic phosphorus content (orthophosphate, expressed in $mg\,L^{-1}$), and COD is the chemical oxygen demand ($mg\,L^{-1}$).

For the bacterioplankton community analysis, 1000 mL water samples were filtered onto a 0.2 μm pore-sized polycarbonate filter (47 mm diameter, Millipore, Boston, MA, USA) on the sampling day. The filters were immediately frozen at − 80 °C until they were needed.

2.3. DNA extraction, PCR amplification and MiSeq sequencing

Microbial DNA was extracted directly from the filter using a Power Soil® DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. The DNA extracts were quantified by the ratios of 260/280 nm and 260/230 nm using a spectrophotometer (NanoDrop Technologies Inc, Wilmington, DE, USA). The V3-V4 regions of the bacterial 16S rDNA gene was amplified (30 μl reaction volume; started from 95 °C for 3 min; followed by 28 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 45 s; and finalized with a 10 min extension step at 72 °C) using primer set 338 F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′), with overhang sequences as adaptors to link to the barcodes at the 5′end for each primer. PCR was performed in triplicate for each sample, and the products were purified using a PCR fragment purification kit (Takara, Japan) and checked using the Quant-iT PicoGreen dsDNA quantification kit (Invitrogen, Carlsbad, CA, USA). The purified products were combined into equimolar ratios for paired-end (PE) library preparation, and 300 bp PE sequencing on an Illumina MiSeq platform (Illumina, San Diego, CA, USA).