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Microeukaryotic biogeography in the typical subtropical coastal waters with multiple environmental gradients

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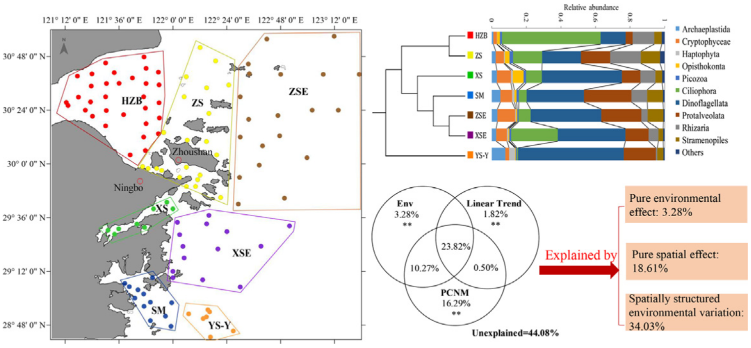
HIGHLIGHTS

- Microeukaryotic communities in subtropical coastal waters were investigated.
- The microeukaryotes were dominantly comprised of *Dinoflagellata* and *Ciliophora.*
- The distribution of top abundant taxa exhibited environment-conditioned features.
- Microeukaryotic α-diversity was associated with phosphorus and suspended particles.
- Spatially-structured local conditions largely shape microeukaryotic biogeography.

GRAPHICAL ABSTRACT



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ABSTRACT

The determinants of microeukaryotic biogeography in coastal waters at a regional scale remain largely unclear. The coastal northern Zhejiang (in the East China Sea) is a typical subtropical marine ecosystem with multiple environmental gradients that has been extensively perturbed by anthropogenic activities. Thus, it is a valuable region to investigate the key drivers that shape microbial biogeography. We investigated microeukaryotic communities in surface waters from 115 stations in this region using 18S ribosomal RNA gene amplicon sequencing. The microeukaryotic communities were mainly comprised of *Dinoflagellata*, *Ciliophora*, *Protalveolata*, *Rhizaria*, *Stramenopiles* and *Cryptophyceae*. The top abundant operational taxonomic units (OTUs) were highly specific for distinct habitat types, exhibiting significant environment-conditioned features; however, the cosmopolitan OTUs were not strongly correlated with the measured environmental variables. Total phosphorus and suspended particles were major environmental determinants of microeukaryotic α-diversity. Environmental variables, particularly temperature, salinity, pH and silicate concentration, were strongly associated with the microeukaryotic community composition. Overall, environmental and spatial factors explained 55.92% of community variation in total with 34.03% of the variation shared, suggesting that spatially structured environmental variations mainly conditioned the microeukaryotic biogeography in this region. Additionally, dispersal limitation, as indicated by the great pure spatial effect and distance-decay pattern, was another important factor. In summary, our results reveal that spatially structured environmental variation and dispersal limitation mainly

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conditioned the microeukaryotic biogeography. The results may provide useful distribution patterns of microeukaryotes to determine sources of microbes from marine ecosystems that may facilitate the utilization of coastal resources.

1. Introduction

Microeukaryotes are a keystone of the marine microbial food web due to their sheer abundance and phylogenetic and functional diversity (de Vargas et al., 2015). The microeukaryotes, such as phytoplankton, may carry out roughly half of the primary production, while protists and fungi may function as consumers, decomposers and parasites, and all of which can significantly regulate biogeochemical cycling (Priscu, 1995). Traditionally, surveys of microeukaryotic diversity have largely relied on morphological taxon identification, which have demonstrated that microeukaryotic diversity could be influenced by multiple biotic factors (including prey availability and top-down grazing) and abiotic factors (including light, temperature, salinity, nutrients and spatial factors) (Kim et al., 2014; Sherr et al., 2007), but the relative importance of these factors is still unclear, especially in coastal areas. Given the contributions of microeukaryotes to the ecosystem's stability, resilience, and function, and the continued provision of coastal ecosystem services (Iii et al., 2000), it is essential to explore the dominant factors controlling microeukaryotic community composition in coastal areas.

Advances in microbiological and molecular biological techniques have proved that microeukaryotic community structures shift rapidly with environmental variables, geographic locations, depth and season (Caron et al., 2012; Piwosz and Pernthaler, 2010). However, the dominant determinants of microeukaryotic biogeographic patterns, namely, whether they are fundamentally governed by environmental conditions or spatial factors, remain to be clarified. Environmental variables have been shown to be critical to bacterial communities (Van der Gucht et al., 2007; Wang et al., 2015b) and microeukaryotic communities (Liu et al., 2013; Wojciechowski et al., 2017). Environmental variables such as nutrient concentrations can influence microeukaryotic community compositions because they are essential for the growth and development of microeukaryotes, and different microeukaryotes are adapted to their optimal growth concentrations. Furthermore, the nutrient, which can influence the photosynthesis of autotrophs, can also affect microeukaryotic community compositions. Spatial factors are another key determinants, which may play pivotal roles over restricted spatial scales in all organisms, and similarities of community compositions decline with increasing distance among habitats (Shurin et al., 2009). Due to the fact that microbial communities are largely connected among local environments via dispersal, there has been a complex interplay between environmental conditions and spatial factors (Amaral-Zettler et al., 2011; Ragon et al., 2012). Studies of estuarine ecosystems, freshwater lakes and marine environment have highlighted that both environmental selection and spatial factors can shape microeukaryotic diversity (Filker et al., 2016; Lallias et al., 2015; Zhang et al., 2018). Some recent studies suggest that both environmental and spatial factors need to be considered when investigating the microbial biogeography (Liu et al., 2015; Zhang et al., 2018). Thus, it is a major challenge to disentangle the relative importance of these factors in conditioning microbial community structure, as the power of these factors and their complex interactions depends on the scales of geographic and environmental gradients.

The coastal northern Zhejiang (close to the Yangtze River and Qiantang River estuary in the East China Sea) is a typical subtropical coastal area including a large estuary-bay area, an archipelago, and a nature reserve with multiple environmental gradients (Wang et al., 2015a). Thus, the coastal northern Zhejiang is an ideal marine ecosystem to study the roles of environmental conditions and spatial factors, as mentioned above. Moreover, the planktonic bacterial biogeography in this region is strongly influenced by spatially structured environmental gradients (Wang et al., 2015a), but the patterns of microeukaryotic biogeography remain unclear. In the present study, we aimed to explore the biogeographic patterns of microeukaryotic communities in surface waters using the 18S ribosomal RNA (rRNA) gene amplicon sequencing. Compared with the global scale investigation (de Vargas et al., 2015; Lie et al., 2014), we conducted a denser sampling strategy to achieve higher coverage over a relatively small marine ecosystem. Ultimately, we collected 115 surface water samples from seven different zones (Fig. 1) and aimed to (i) investigate the distribution pattern of microeukaryotes in a typical coastal area at an intermediate spatial scale; (ii) study the relationship between environmental variables and microeukaryotic community composition; and (iii) assess the relative importance of environmental and spatial factors in determining the microeukaryotic community structure.

2. Material and methods

2.1. Sampling and physicochemical analyses

Surface water samples were collected in March 2015 from 115 stations across seven coastal zones, including Hangzhou Bay (HZB), Zhoushan Islands (ZS), the eastern Zhoushan Islands (ZSE), Xiangshan Bay (XS), the eastern Xiangshan (XSE), Sanmen Bay (SM) and Yushan Reserve (YS-Y), which geographically cover the main marine area of coastal northern Zhejiang (Fig. 1, Dataset S1). Stations in the HZB were located at the estuary of Qiantang River. Stations in the ZS and ZSE were located at the fishery. XS and SM are semi-enclosed bays with intensive agricultural pollution. YS-Y is a nature reserve. Water samples were collected from the surface layer (0.5 m) using a 5 L water sampler on the deck. Approximately 600 mL of seawater were pre-filtered by a 100 μm-pore size Nylon filter, and next the water samples were filtered through 0.2 μm-pore size membrane (47-mm diameter polycarbonate, Millipore, USA). After that, the membranes were frozen at −20 °C on board and transferred to −80 °C in the laboratory. To prevent contamination between stations, water sampler and filtration system were washed with sterile water carefully before water collection and filtration. Water temperature, salinity and pH were measured in situ. Dissolved oxygen (DO), total nitrogen (TN), chemical oxygen demand (COD), total phosphorus (TP), suspended particles (SP), phosphate, silicate, nitrate, nitrite and ammonium were measured using standard methods (AQSIQ, 2007). The sampling map and Kriging maps were all made by Surfer 13 (Golden Software).

2.2. DNA extraction, PCR amplification and MiSeq sequencing

Environmental DNA was extracted from the samples using a Power Soil DNA Isolation Kit (MO BIO, USA) according to the manufacturer's instructions, and DNA concentrations were measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher, USA). Then, the samples were submitted for library preparation and sequencing to the MajorBio Co., Shanghai. Briefly, the V4 region of the 18S rRNA gene was amplified using specific primers 3NDF (5′-GGCAAGTCTGGTGCCAG-3′) and V4_euk_R2 (5′-ACGGTATCT(AG)ATC(AG)TCTTCG-3′) (Bråte et al., 2010). For each sample, an aliquot of 10 ng of purified DNA template was amplified in triplicate with a 20 μL reaction system under the following procedures: denaturation at 95 °C for 3 min, followed by 32 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final extension of 72 °C for 10 min.