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Changes in community structure of active protistan assemblages from the lower Pearl River to coastal Waters of the South China Sea

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Abstract

Protists make up an important component of aquatic ecosystems, playing crucial roles in biogeochemical processes on local and global scales. To reveal the changes of diversity and community structure of protists along the salinity gradients, community compositions of active protistan assemblages were characterized along a transect from the lower Pearl River estuary to the open waters of the South China Sea (SCS), using high-throughput sequencing of the hyper-variable V9 regions of 18S rRNA. This study showed that the alpha diversity of protists, both in the freshwater and in the coastal SCS stations was higher than that in the estuary. The protist community structure also changed along the salinity gradient. The relative sequence abundance of Stramenopiles was highest at stations with lower salinity and decreased with the increasing of salinity. By contrast, the contributions of Alveolata, Hacrobia and Rhizaria to the protistan communities generally increased with the increasing of salinity. The composition of the active protistan community was strongly correlated with salinity, indicating that salinity was the dominant factor among measured environmental parameters affecting protistan community composition and structure.
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Keywords: Community structure; Estuary; High-throughput sequencing; Microbial eukaryotes; Salinity gradient; 18S rRNA

Introduction

Protists play important roles in structuring marine ecosystems and global biogeochemical cycles (Azam et al. 1983; de Vargas et al. 2015; Sherr and Sherr 2002; Zubkov and Tarran 2008). Numerous studies have shown that due to various environmental factors, the community structure and diversity of protists varies both spatially and temporally (Caron et al. 2011; Lallias et al. 2015; Lie et al. 2013). For a better understanding of the primary ecological roles played by protists, their biogeographical distribution and response to changing environments represent fundamental questions that need to be addressed (Caron et al. 2016).

The South China Sea is one of the largest semi-enclosed marginal seas (Hu et al. 2000). With large inputs of fresh water and nutrients input from the Pearl River and oceanic water intrusion, the northern South China Sea is characterized by sharp environmental gradients over small spatial scales (Ning et al. 2004). It is thus an ideal environment for examining the spatial variation in microbial community composition and the mechanisms by which this is controlled.

Traditional microscopic methods have significant limitations for the study of protists because: (i) species identification is restricted to those organisms with well-

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documented morphological characteristics; (ii) they require large amounts of resources in terms of time and effort; (iii) species that are rare, cryptic and/or present in low abundance are frequently overlooked; and (iv) a wealth of taxonomic knowledge and expertise is needed (Eland et al. 2012). Given the wide range of protistan groups that exist, these requirements are seldom met. Fortunately, molecular sequencing methodologies have overcome these difficulties resulting in the discovery of previously unexpected patterns of distribution and high diversities of protists (Logares et al. 2014; López-García et al. 2001; Massana et al. 2004). In recent years, the development of 18S rRNA (and its gene)-based high-throughput sequencing technology has enabled us to quickly obtain hundreds of thousands to millions of environmental sequences at low cost, allowing detailed studies of microbial community structure (de Vargas et al. 2015; Hu et al. 2016; Lie et al. 2013), evolutionary relationships (Dunthorn et al. 2014; Sun et al. 2016), and microbiological and ecological relevance (Lallias et al. 2015; Lima-Mendez et al. 2015). In environmental surveys, the 18S rRNA gene has been commonly used as a marker to determine protistan community structure in various aquatic habitats (Guillou et al. 2008; Logares et al. 2014). However, extracellular DNA or DNA from dead/dormant cells can distort the results because this cannot be discriminated from the DNA of living/active cells using DNA-based sequencing alone. Compared with DNA, RNA is much less stable in extracellular conditions. Therefore, most RNA recovered from environmental samples originates from living cells. Estuarine systems are hotspot for a series of biogeochemical processes, harboring a variety of microbial communities adapted to wide ranging of salinity fluctuations (Attrill and Rundle 2002; Bazin et al. 2014a; Vigil et al. 2009). Studies have shown that a large number of dead cells/extracellular DNA exist in estuarine habitats (DeFlaun et al. 1987). Ensuring that nucleic acids originate from living cells is particularly relevant when investigating active protist communities in such environments. Nevertheless, most previous studies of protists were based on DNA sequencing (de Vargas et al. 2015; Decelle et al. 2014; Xu et al. 2017a) and only a few have employed the RNA-based sequencing (Charvet et al. 2014; Hu et al. 2016; Logares et al. 2014; Massana et al. 2015; Xu et al. 2017b). Studies of protist communities in estuarine environments involving RNA sequencing are even rarer (Sun et al. 2017).

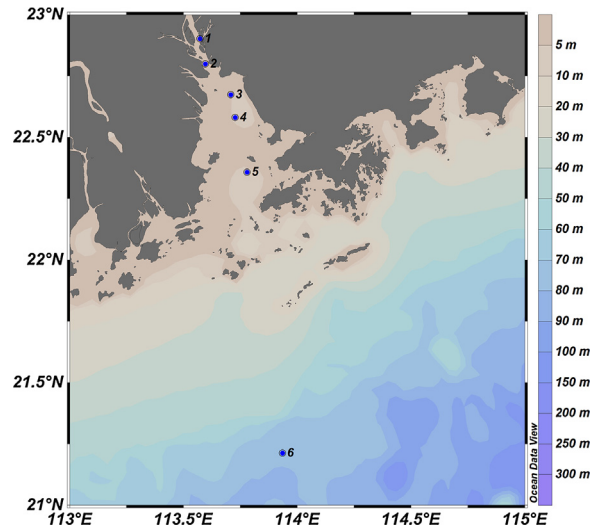

Fig. 1. Geographic locations of the sampling sites along the Pearl River estuary and in the northern South China Sea.

In the present study, we investigate the active protistan communities that occur in the Pearl River estuary, southern China, by high-throughput amplicon sequencing of the V9 region of the 18S rRNA. The main aims are to evaluate the composition of active protistan assemblages along salinity gradients and to increase understanding of the factors that shape their community structure.

Material and Methods

Sample collection and environmental parameters

Fig. 1 shows the locations of the sampling sites in the Pearl River estuary and a near-shore open water site of the SCS. A total of six surface water samples (sites 1-6) and two subsurface samples at sites 1 and 5 were collected from 27 July 2013 to 4 August 2013. Sample IDs corresponding to the sites from where they were collected are shown in Table 1. Samples were collected on Millipore filters (pore size, 0.8 μm) from 1 liter of seawater after passing through 200 μm mesh-size pre-filter in order to reduce the contribution of metazoa to nucleic acids

Table 1. Sampling sites information and physical parameters measured.

Sample ID	Site	Latitude	Longitude	Depth (m)	Salinity (‰)	pH	Temperature (°C)	DO (mg/L)
1S	1	22°54’6.66”	113°34’21.60”	2	0	6.1	29.29	1.2
1D	1	22°54’6.66”	113°34’21.60”	8	0	6.2	29.01	0.8
2S	2	22°47’56.2”	113°35’46.80”	1	1	7.2	30.67	5.6
3S	3	22°40’23.39”	113°42’30.41”	1	1	8.1	30.89	8.1
4S	4	22°34’51.24”	113°43’39.28”	1	4	8.1	30.66	8.2
5S	5	22°21’27.48”	113°46’48.12”	2	18	8	28.00	6.7
5D	5	22°21’27.48”	113°46’48.12”	8	26	8.2	27.63	6.3
6S	6	21°12’42.00”	113°56’09.00”	2	34	8.3	29.09	6.6

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