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The spatial distribution of benthic foraminifera in the Pearl River Estuary, South China and its environmental significance

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ABSTRACT

Thirty surface sediment samples were collected from the Pearl River Estuary, South China, and benthic foraminifera were analyzed in order to understand the relationship between foraminiferal assemblages and environmental parameters. Multivariate analyses showed that the foraminferal assemblages (i.e., abundance and diversity) are correlated with the hydro-sedimentary gradients within the estuary. In addition, the dominant faunal composition seems to be largely influenced by food availability and trace metal contamination in surface sediments. A comparison with historical data from 1980s demonstrated that the foraminiferal abundance and diversity in the lower estuary have dramatically decreased over the last three decades, together with a significant shift in the dominant species. This is most likely due to the cumulative impacts of eutrophication and Cu contamination caused by human activities in the Pearl River basin. This work confirms the value of benthic foraminifera as bio-indicators in polluted estuarine environments.

1. Introduction

Benthic foraminifera are useful in environmental studies because they respond sensitively to environmental changes due to their life-cycle of some weeks-months (Sharifi et al., 1991; Scott et al., 2001; Murray, 2006). Compared with other ecological bio-indicators, foraminifera are very abundant in the sediments of most marine and transitional environments. Most species have a hard shell called "test" which may be well preserved in the sedimentary records, and therefore be more suitable for paleo-environment reconstruction (Alve, 1991; Scott et al., 2001; Mojtahid et al., 2016). The distributional pattern of recent benthic foraminifera in marine and transitional environments is regulated by a diverse range of physical, chemical and biological parameters, such as salinity, sediment characteristics, oxygen and food availability (Murray, 2006 and references therein). As variations in environmental parameters separate species specific habitats, making particular microhabitats attractive for some species, but uninhabitable for others, associations of benthic foraminifera can be used as indirect indicators for modifications of environmental parameters (Kaiho, 1994; Le Cadre and Debenay, 2006; Nikulina et al., 2008). However, the reliable use of this proxy should be based on an improved understanding of foraminiferal ecology and their response to major environmental parameters (Kramer and Botterweg, 1991; Ernst et al., 2006; Murray, 2006; Li et al., 2014, 2021).

Estuaries act as transitional ecotones between terrestrial and marine systems. They are subject to large physical, chemical and biological gradients, and are strongly impacted by a range of human activities such as nutrient-enhanced eutrophication and trace metal pollution. As a result, understanding the foraminiferal response to certain major environmental parameters presents a challenge, since they may be influenced by both natural and anthropogenic processes (Debenay et al., 2001; Murray, 2006). In recent decades, benthic foraminifera are increasingly used as bio-indicators in estuaries and coastal oceans, because of the rising demand of public actors to find urgently an integrative tool to monitor the health of estuaries. Additionally, time series and population dynamics studies of live (Rose Bengal stained) benthic foraminifera are conducted in some human-perturbed estuaries

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(Gustafsson and Nordberg, 2000; Leorri et al., 2008; Goineau et al., 2011; Martins et al., 2016; Li et al., 2021).

The Pearl River Estuary (PRE), a large subtropical estuary located in southern China, has been a site of extensive investigation into benthic bio-indicators. Previous studies on modern foraminifera are limited, restricted to a few abiotic parameters and mostly focused on sediment characteristics. Li (1985), for the first time, gave a preliminary view of distribution patterns of foraminiferal communities, focusing on the possible influence of sedimentary dynamics. Luo et al. (2001) and Wu et al. (2015) performed more precise studies providing a good preliminary taxonomic investigation of both dead and living faunas in the estuary. Their studies, together with Li et al. (2015), suggested that the foraminiferal assemblages are mainly governed by the hydrodynamics (i.e., salinity and currents) and/or sedimentary substrate. Conversely, more recent studies conducted by Li et al. (2013, 2014) reported that trace metal contaminant, not considered in previous studies, may have played a critical role in controlling foraminiferal faunas in the PRE and the adjacent coastal waters. However, without a complete faunal and environmental data set, it is difficult to elucidate the natural and anthropogenically-induced impacts on foraminiferal assemblages; in particular the PRE has experienced degraded environments due to the increased human activities in the last 4 decades (Harrison et al., 2008; Dai et al., 2008; Chen et al., 2012).

In this study, we investigated the structure, distribution, and composition of foraminiferal assemblages and their link to the environmental gradients (including a range of physiochemical parameters, sediment features, organic carbon and trace metals) in 30 surface sediment samples collected from the Pearl River Estuary. This study aims to characterize the modern foraminiferal assemblages and to assess its ecological response to the environmental gradients in this complex estuarine environment, with particular focus on the human impacts. Moreover, by comparing our data with the foraminiferal assemblages reported previously, we also intended to investigate the foraminiferal response to environmental changes over the last decades, a period when the human population and economic activities have been dramatically expanded over the Pearl River delta. This research may be considered as baseline for future bio-monitoring studies focusing on the application of benthic foraminifera as indicators of environmental stress in this large perturbed estuary and in transitional environments in general.

2. Materials and methods

2.1. Study area

The Pearl River Estuary (PRE) is one of the most complex estuarine systems in the world, connecting with the northern South China Sea. It is shallow (4.7 m on average) with two deep channels (i.e., East and West Channels) and three shoal regions (i.e., East, Middle and West Shoals). The PRE is divided into three sub-regions (i.e., upper, middle and lower estuary) by the line from Neilingding Island to Qi'ao Island, and from Macau to Lantau Island (Fig. 1). The flow of freshwater is dominated by the Pearl River system, the second largest river in China in terms of freshwater discharge (350×10^9 m^3/yr) (Harrison et al., 2008). About 80% of the total annual discharge and more than 90% of the suspended sediment from the Pearl River is discharged during the wet season (from April to September), with maximum river discharge occurring in July (Zhao, 1990). Approximately 80% of sediment load to the estuary is deposited within the PRE, and the remaining being transported to the South China Sea (Wai et al., 2004).

The hydrodynamics conditions and sedimentation in the estuary is influenced complicatedly by the seasonal river discharge, tide and monsoonal winds (Harrison et al., 2008). In general, the river discharge dominates at the surface as southwestern flow, while the tidal inflow occurs in the lower near-bottom layer and enters the PRE from the southeast through deep channels. As a result, residual current in this subtropical estuary is characteristic of a typical two-layer estuarine circulation (Zhao, 1990). In general, higher current velocities (0.5–0.8 m/s) are associated with tidal channels. The relative intensity and movement of freshwater and seawater as well as the interactions between them are crucial factors controlling the sedimentation in the estuary (Zhou et al., 2004). Except for occasional destruction by storms and typhoons, sediments in the PRE are well preserved under weak tidal regime (Owen, 2005).

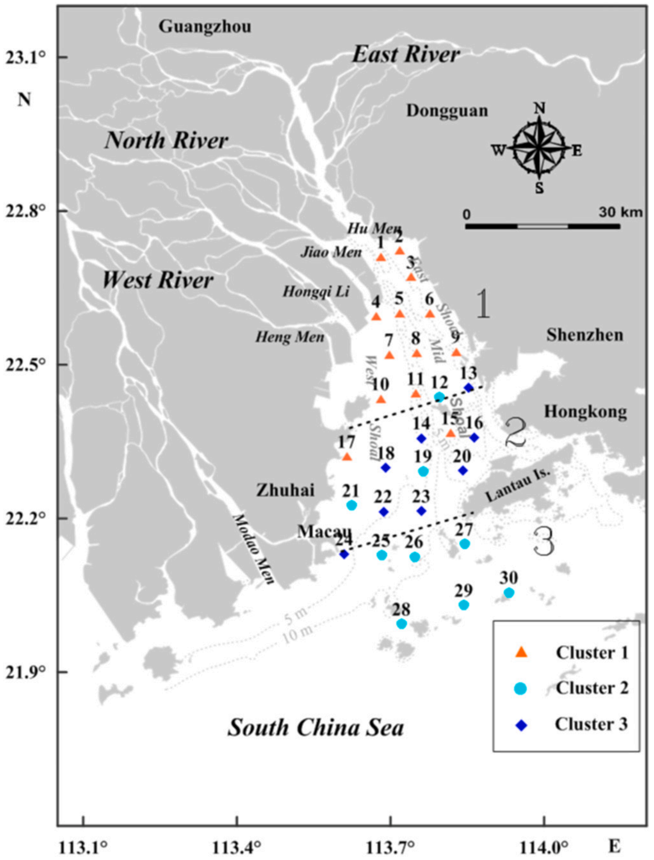

Fig. 1. Map of the study area showing the location of sampling sites. The Pearl River Estuary is divided into three sub-regions: 1. upper estuary; 2. middle estuary; 3. lower estuary. The 5- and 10-m isobaths are shown as thin dashed lines.

Like most major estuarine ecosystems worldwide, the PRE has been increasing impacted by anthropogenic activities during the past decades, including the enhanced agricultural activities, rapid population growth and economic development in the Pearl River Delta region and Hong Kong. As a consequence, many environmental issues have emerged, among which eutrophication, harmful algal blooms and bottom water hypoxia have attracted more attention for the past few decades (Huang et al., 2003; Harrison et al., 2008). In addition, trace metal in surface sediments of the PRE have generally increased over recent decades and have become a serious pollution problem in this region (Chen et al., 2012). These issues could result in changes in the biotic assemblage composition in marine deposits. In addition, the reduced sediment flux to the estuary due to the construction of more than 8600 reservoirs over the Pearl River basin from l990s (Wu et al., 2016) may also cause a change in the geochemical characteristics in the surface sediments on long-term scales.

2.2. Sampling

Surface sediment samples from 30 stations were collected from the Pearl River Estuary in August 2011 using a Van Veen grab sampler, at depth ranging between 3.0 and 25.5 m (Fig. 1 and Appendix A). These stations were chosen because they were less affected by the continuous dredging carried out to maintain navigation channels. On the boat, the grab is carefully opened in a container where the sediment is deposited in its initial position (Debenay et al., 2001). The uppermost sediment layer of sediment (roughly 2 cm) was scraped off. The sedimentation rates calculated for the area range between 1.0 and 2.0 cm/yr, therefore, the sediment samples should have accumulated during the past 1–2 years (Chen and Luo, 1991). Sediment samples were divided into two parts, with one portion (~50 g) used to determine the foraminifera distribution and the other one used for geochemical analyses. Sediment sub-samples used for foraminiferal analysis were preserved in 95% ethanol containing 1 g/L Rose Bengal, a commonly used stain for live foraminifera identification (Murray, 2006), immediately after retrieval, and kept cool until on-shore treatment. In the laboratory, samples were stored in dark for 14 days, in order to provide the time for thorough staining of all living foraminifera (Walton, 1952; Schönfeld et al., 2012). The other sub-samples were stored in sealed plastic bags and transported on ice to the laboratory where they were stored at −20 °C until further analysis.

2.3. Physiochemical data

At each station, temperature and salinity were measured in situ with a multi-parameter sensor (YSI 6600). Water samples were collected from the bottom water (~1 m above the sediment) and were analyzed for oxygen content in triplicate using the standard Winkler method (Grasshoff et al., 1983). Bottom water samples for nutrients and chlorophyll *a* (Chl a) were collected by filtered through a glass fiber filter (GF/F, 47 mm) and subsequently samples were stored at −20 °C until analysis. Concentrations of dissolved nitrate were measured by the Cu-Cd column reduction method (Wood et al., 1967), with a detection limit of 0.05 μmol/L. Chl a concentrations were determined using a Fluorometer (Turner Designs Model 10) after extraction in 90% acetone at 4 °C for 24 h. Note that the concentrations of DO and dissolved nitrate in the bottom layer have been reported by Ye et al. (2013).

2.4. Geochemical analyses

Sediment grain-size analysis was performed according to the method described by Quintino et al. (1989). In short, the organic matter was firstly removed using H_2O_2. Subsequently, the residual samples were separated using dry and wet sieving through a Wentworth series of meshes (63, 125, 250, 500, 1000 and 2000 μm). In this study, particle sizes with diameter smaller than 63 μm, 63 μm–2 mm and >2 mm were regarded as mud/clay, sand and gravel fractions. The pH of the sediment was measured by a Thermo Orion 3 Star pH Benchtop, after adding KCl solution to the sediments (Gao et al., 2015).

Sediment samples were freeze-dried, homogenized and partitioned into subsamples. Total organic carbon (TOC) and total nitrogen (TN) contents were measured in duplicate on a CHNS elemental analyzer (Vario EL III, Germany), after treatment with 5% HCl to remove carbonate at the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences. The reproducibility was satisfactory with an average relative standard deviation (RSD) for replicate analyses of 0.9% for TOC and 1.3% for TN. Weight percentage of TC were determined without acidification by combustion on the elemental analyzer, and the quantity of inorganic carbon (IC) was determined by the difference of TC and TOC. The $CaCO_3$ (carbonate) content was calculated from the IC content by multiple the IC by 8.33, and assuming that all IC is present as $CaCO_3$ (Jia et al., 2013). Analytical procedures for trace elements determinations (Co, Cu, Ni, Pb, Zn, Hg) have been described in Chen et al. (2012) and Yin et al. (2015). Quality control was performed using analytical blanks and certified reference materials (BCR 580 for total Hg and NIST 1646a for other trace elements). The metal concentrations in the blanks were <1% of the sediment samples and the relative standard deviations of the replicate samples were lower than 10% (Chen et al., 2012). The detection limits were 0.5 ng/g for Hg and 1.0 to 2.0 μg/g for other trace metals (i.e., Cu, Pb, Zn, Co and Ni). The concentrations of perfluoroalkyl substances (PFASs) have been reported in details by Gao et al. (2015).

2.5. Foraminiferal analyses

In this study, the Rose Bengal stained sediments were firstly wet sieved over a 2000 μm mesh to remove mollusks shells or big pebbles, and then gently sieved through a 63 μm mesh, after which the residuals were oven dried at 50 °C. The live (stained) and dead (unstained) individuals were counted separately and studied under a stereoscopic binocular microscope using reflected light at the Department of Earth Sciences, Sun Yat-sen University. The taxonomic analysis of benthic foraminifer species was based on the Li (1985), Wang et al. (1988), Huang and Yim (1998) and Garrett (2010) classification. In the present work, we report the total concentration of benthic foraminifer shells (number of specimens per 50 g sediment) and the relative abundance (%) of the most abundant taxa.

2.6. Mapping and statistical analysis

Maps of the spatial distribution of benthic foraminifera and environmental parameters were performed using Surfer v.11 (Golden Software, Golden, CO, USA). A Q-mode cluster analysis based on the absolute abundance of living and dead foraminifera as well as the percentages of common species (Ward's method) was used to determine the similarity among sampling sites.

A Detrended Correspondence Analysis was calculated to determine whether the distribution of species was linear or unimodal (Leps and Smilauer, 2005). A linear response was observed, so we performed Redundancy Analyses (RDA) to evaluate the influence of environmental variables on foraminiferal distribution, by using CANOCO v5 statistic software (Microcomputor Power Inc., Ithaca, NY, USA). Input for RDA consisted of assemblage/taxon data and environmental data. Only foraminiferal species with relative abundances more than 5% in at least one site, and were present in at least 10 stations were retained in the analyses. Meanwhile, Pearson correlation analysis was also carried out to studying the relationship between the environmental parameters and foraminiferal characteristics by the software SPSS 16.0 (IBM SPSS, Chicago, MI, USA). Correlations were considered significant for $p < 0.05$.

3. Results

3.1. Abiotic data

The physiochemical, sedimentological and geochemical data of the 30 sampling stations are presented in Fig. 2. These data provide a general environmental pattern of the Pearl River Estuary. In general, we observe an overall increase in bottom water salinity, but decreases in temperature and nutrient concentrations (i.e., nitrate and phosphate) towards the lower estuary. For the bottom DO, relatively high contents (6–8 mg/L) were recorded in stations located in the middle estuary, where the highest concentrations of algal biomass (*Chl a*) were measured (5–11 μg/L). Low DO concentrations (<3 mg/L) were found at the river mouth (Sta. 1) and the outmost stations (Sta. 28–30), which is attributed to the intensive organic matter degradation and strong stratification of the water column (Ye et al., 2013). The sedimentary pH presented relatively high values in the lower estuary (7.8–8.2), and the minimum values (6.7–7.4) were observed near the river mouth (Sta. 1–5) (Fig. 2f).

Sedimentary organic carbon (SOC) content ranged between 0.30% and 1.38%, with a declining trend from land to sea. The lowest contents (<0.6%) occurred in sandy sediments (Stations 2 and 29) and the highest ones in fine sediments (Stations 4 and 24). The carbonate content is comprised between 0.31% and 3.79%. The minimum contents

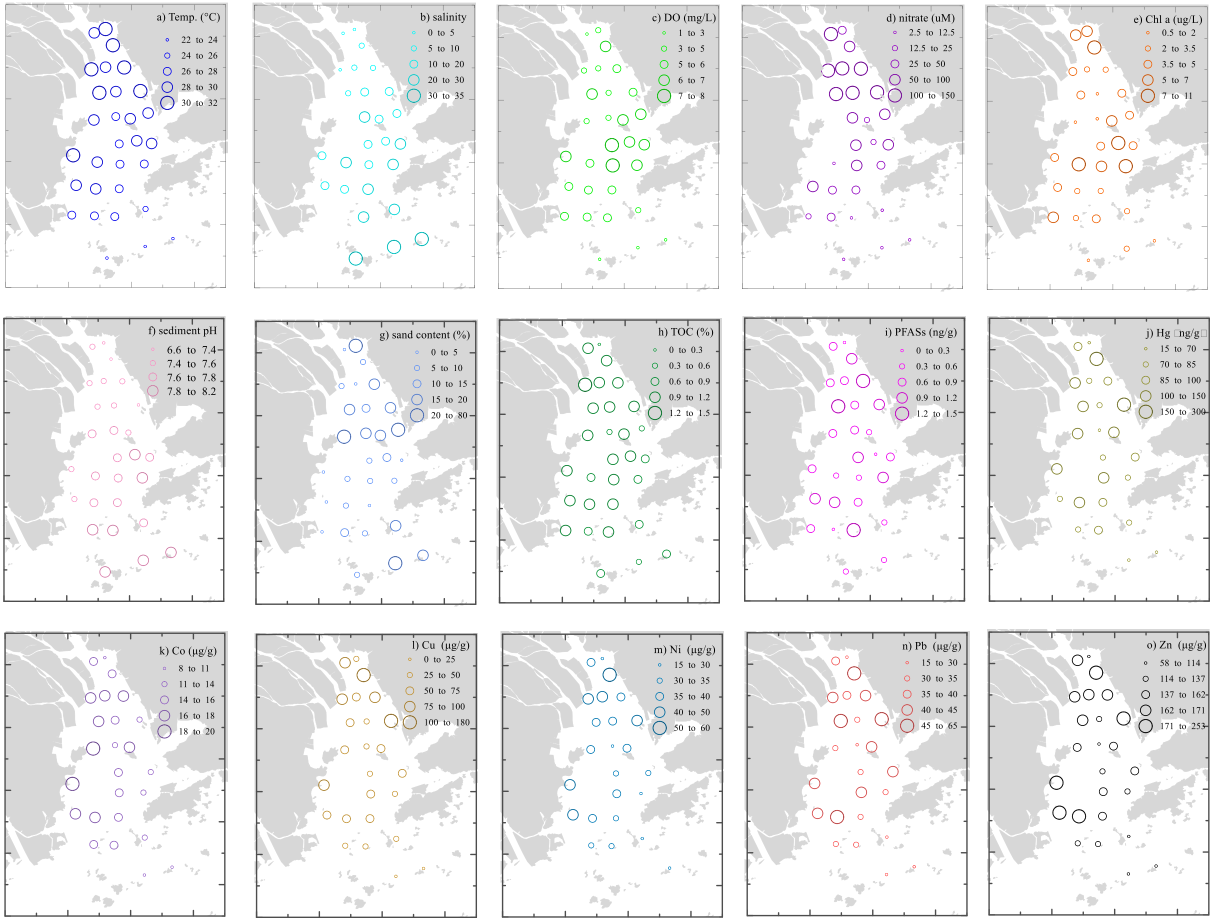

Fig. 2. Spatial distributions of (a–e) bottom water hydro-chemical, (f–g) sedimentological and (h–o) geochemical parameters in the Pearl River Estuary.

(<1.0%) were found in the upper and middle estuary, and the maximum ones (>2.5%) in the lower estuary (Stations 28–29) and some inner nearshore stations (Stations 4 and 13). Results of trace metals (Co, Cu, Ni, Pb and Zn) and total Hg concentrations are presented in Chen et al. (2012) and Yin et al. (2015). In short, trace metal concentrations had a large spatial variability within the estuary, with high values observed in the upper estuary, mainly due to the impact of urban waste, shipyards and industries. All metals are highly correlated with each other and positively correlated to the fine sediment fraction but negatively to sand fraction (Chen et al., 2012; Yin et al., 2015). Perfluoroalkyl substances (PFASs) have a distributional pattern similar to that of TOC, with high concentrations recorded in sites with fine sediments but low concentrations in grained sediments (Gao et al., 2015).

3.2. *Benthic foraminiferal distribution*

In the present study, living and dead foraminifers were found in most sampling sites of the Pearl River Estuary, and there was a general seaward increase in foraminiferal density (FD) and species richness (Fig. 3).

3.2.1. *Living assemblages*

In the study area, a total of 29 living species of benthic foraminifera and 15 genera left on open classification were identified, among which 19 species showed a relative abundance larger than 5% in at least one sample. The living foraminiferal density ranged from 0 to 2112 tests/50 g in the whole estuary (Fig. 3). The absent of living specimens near the river mouth may be due to the unstable environment and/or high levels of pollution (Luo et al., 2001; Wu et al., 2015), which will be discussed later. The maximum foraminiferal density (2112 ind./50 g) was found at a water depth of 19.5 m (Sta. 29). Out of the 29 identified species, *Quinqueloculina akneriana* (35.3% on average) and *Ammonia beccarii* (13.8% on average) are the most abundant species in both the middle and lower estuary, followed by *Quinqueloculina* cf. *tropicalis*, *Rotalidium annectens* (also previously reported as *Ammonia annectens* and *Cavarotalia annectens*), and *Hanzawaia mantaensis* (3.6%, 3.0% and 2.0% on average). Our data are consistent with previous results in the PRE (Luo et al., 2001; Wu et al., 2015), particularly that of Wu et al. (2015) in which they sampled one year (September−October 2010) prior to our study. The highest relative abundances of live *Q. akneriana* and *A. beccarii* were recorded from the upper and middle estuary, respectively.

3.2.2. *Total (living plus dead) assemblages*

Compared with the living counterpart, almost all study samples contain relatively abundant and well preserved dead foraminifera. For the total benthic foraminifera, a total of 76 species (29 live and 47 dead species) were identified belonging to 35 genera and the abundance ranged from 0 to 20,448 individuals per 50 g sediment (Fig. 3). The relative abundance of the recognized species varied from station to station with only 25 species showing relative abundance exceeding 5% of the total assemblage in at least one sample. The foraminiferal community is mainly dominated by calcareous hyaline and miliolid species with the minor occurrence of agglutinated taxa (<2.5%). The most abundant species was *A. beccarii* (15.2%), followed by *Q. akneriana* (13.5%), *Elphidium advenum* (10%) and *R. annectens* (6.5%) (Fig. 4). Among the rest of species, *Hanzawia nipponica*, *Rosalina bradyi*, *Florilus decorus*, *Ammonia compressiucula* and *Ammonia pauciloculata* were

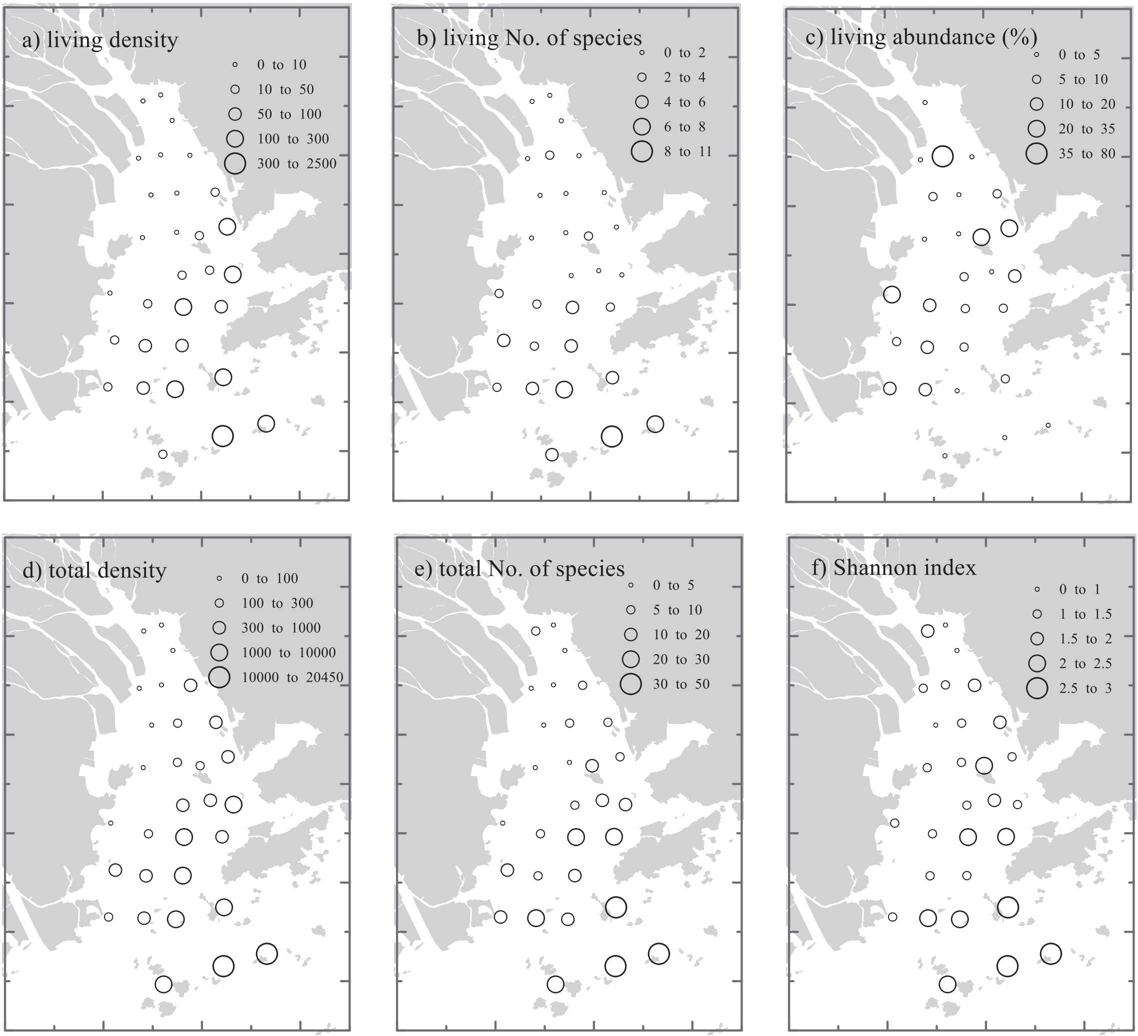

Fig. 3. Spatial distribution of benthic foraminiferal parameters per 50 g sediment: a) living density; b) living species richness; c) relative abundance of living assemblage to total assemblage; d) total foraminiferal density; e) total species richness; f) Shannon index. The size of circle is proportional to the values of the ecological indices.

present more regularly.

It is worth mentioning that the population dynamics (i.e., foraminiferal density and diversity) of total assemblage had a similar distributional pattern of as the living assemblage (Fig. 3). However, the living foraminifera in the upper and middle the estuary were low in abundance and diversity, and it accounted for only a small percentage (<20%) of the total assemblage in most regions. According to Fatela and Taborda (2002), in order to assess the diversity of foraminifera, it may be sufficient to count about 100 specimens in estuarine and coastal environments where the microfauna consist of a few species. As a result, only the total foraminiferal assemblage greater than 100 individuals will be considered in the statistical analyses in the following.

3.2.3. Cluster analysis

In order to classify the estuary with respect to the spatial distribution of benthic organisms, the foraminiferal data (i.e., the FD and species richness of both living and total assemblage, and relative abundance of the most common species) was subjected to cluster analysis (CA, Q-mode). Three major clusters (Cluster 1–3) were identified (Fig. 5). The first major cluster was comprised of stations located in the upper estuary, which was characterized by low FD (<700 and <20 individuals for total and live foraminifera) and diversity (<10 species) of foraminifera. The major taxa in this zone were *A. beccarri*, *R. annectens* and *E. advenum*. The second cluster with high FD (>1000 individuals) and diversity (10–39 species) representing sampling sites in the lower estuary and contained the dominant taxa of *A. beccarri*, *H. nipponica*, *A. compressiuscula* and *E. advenum*. The third cluster was characterized by moderate levels of FD and diversity representing stations in the middle estuary, and the characteristic species included *Q. akneriana*, *A. beccarri*, *R. annectens* and *E. advenum*. A comparison of the distribution of these clusters with the physiographic zonation of the PRE seems to indicate that foraminiferal distribution is mainly controlled by the hydro-sedimentary features, which will be further addressed in the Discussion section.

3.3. Relationship between benthic foraminifera and environmental variables

As indicated by the Pearson correlation analysis, the total absolute densities and species richness of foraminiferal assemblage in the study area appeared to be correlated positively with pH, salinity and depth, but negatively with other physio-chemical parameters like temperature and DO contents (Table 1). Moreover, the foraminiferal abundance shows a negative correlation with some geochemical variables in surface sediments (i.e., TOC and trace metals). Lastly, most species did not display significant relationship with bottom-water properties and most

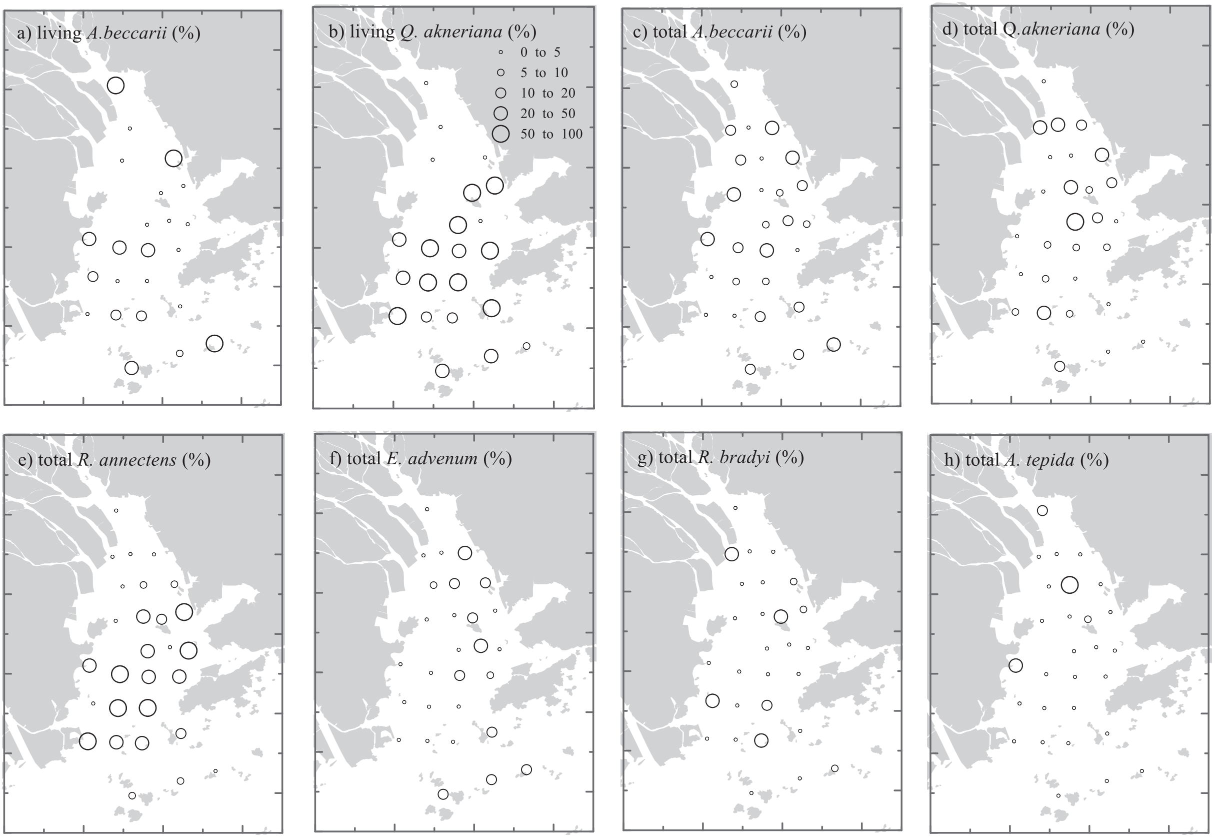

Fig. 4. Spatial distributions of the relative abundances of the six most common species: The size of circle is proportional to the values of the foraminiferal species.

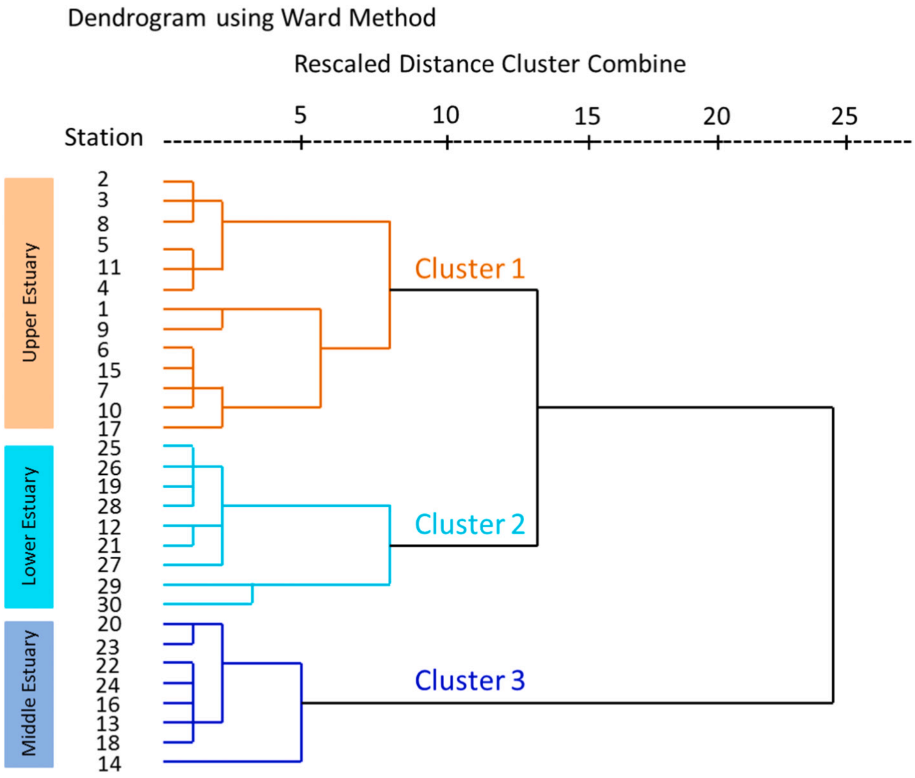

Fig. 5. Q-mode cluster diagram of sampling stations showing the presence of 3 major cluster groups.

Table 1

Pearson correlation matrix for the benthic foraminifera and environmental parameters in bottom waters and surface sediment of the Pearl River Estuary.

	Temp	Salin	DO	Nitrate	Chl a	Sand	Mud	pH	Chlorine	TOC	$CaCO_3$	PFASs	Co	Cu	Ni	Pb	Zn	Hg
Live abund	**−0.50***	0.40	**−0.52***	−0.16	−0.19	**0.78****	**0.79****	**0.59****	**0.47***	**−0.60****	0.24	−0.31	**−0.68***	−0.41	**−0.69****	**−0.54***	**−0.58****	**−0.54***
Live species	**−0.73***	**0.72****	**−0.52***	−0.13	0.17	0.33	**0.66****	**0.69****	0.40	−0.35	−0.02	−0.34	**−0.53***	**−0.47***	**−0.59****	**−0.48***	**−0.48***	**−0.54***
Total abund	**−0.72****	**0.57****	**−0.71****	**−0.42***	−0.26	**0.65****	**−0.60****	**0.63****	−0.01	**−0.60***	0.23	−0.34	**−0.78****	**−0.50***	**−0.74****	**−0.65****	**−0.66***	**−0.66***
Total species	**−0.84****	**0.78****	**−0.61****	**−0.62****	−0.17	0.27	−0.22	**0.71****	0.12	−0.47	0.13	**−0.43***	**−0.75****	**−0.52***	**−0.71****	**−0.61****	**−0.62****	**−0.64****
A. beccarri	0.05	−0.11	0.01	0.14	0.12	0.17	0.05	−0.14	−0.34	0.05	0.24	0.05	−0.11	**0.55***	0.25	0.28	0.30	0.34
R. annectens	0.19	−0.36	0.20	0.30	−0.07	−0.06	0.18	−0.14	−0.12	0.18	0.25	0.13	0.19	0.27	0.23	0.05	0.10	0.18
E. advenum	−0.13	0.02	0.01	−0.09	0.05	0.14	−0.11	0.13	−0.23	−0.11	−0.06	−0.32	−0.18	0.05	−0.10	−0.01	−0.10	−0.04
Q. akneriana	0.02	0.16	0.26	−0.21	**0.51***	−0.15	−0.05	0.11	0.06	−0.05	−0.01	0.08	0.04	−0.08	−0.03	0.07	0.07	−0.01
R. bradyi	0.24	−0.15	0.10	0.02	0.06	−0.16	0.16	−0.25	−0.02	**0.51****	0.07	0.16	0.22	0.14	0.18	0.25	0.20	0.24
A. tepida	0.18	−0.18	−0.03	0.28	−0.28	−0.18	0.19	−0.24	0.22	0.16	−0.35	0.02	0.17	0.02	0.21	0.09	0.12	−0.01
P. granosum	0.07	0.16	0.08	**−0.41***	0.13	−0.24	0.25	−0.08	0.23	0.29	−0.40	0.13	0.20	−0.08	0.09	0.10	0.08	−0.04
A. pauciloculata	−0.19	0.23	−0.27	−0.28	0.25	−0.01	0.03	0.07	0.37	−0.04	0.02	−0.14	−0.18	−0.13	−0.14	−0.16	−0.10	−0.39
F. decorus	**−0.71****	**0.62****	**−0.69****	**−0.44***	−0.36	0.15	−0.12	**0.44***	0.25	−0.37	0.10	−0.29	**−0.58****	**−0.47***	**−0.57****	**−0.60****	**−0.63****	**−0.59****
T. tricarinata	0.01	0.08	0.24	0.36	−0.16	−0.04	0.02	0.13	−0.16	−0.04	−0.08	−0.19	−0.42	0.45	0.10	0.06	0.03	0.12

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

of the geochemical data in sediments. However, the species *A. beccarii* shows a positive correlation with Cu content, whereas F. decorus has a negative correlation with trace metal content (Co, Cu, Ni, Pb, Zn and Hg). In addition, the species *Q. akneriana* is positively correlated with *Chl a* concentration in the water column (Table 1).

In order to assess the influence of measured environmental parameters on the assemblage/taxon in more detail, RDA was carried out between the foraminiferal data (total absolute densities and percentages of the common species) and environmental variables. The results showed that the first two major axes accounted for 72% of the total variance of assemblage/environment relations. The distributions of foraminiferal abundance and diversity as well as species *F. decorus* were positively correlated with pH, water depth, salinity, and negatively correlated with trace metal concentrations, TOC content, DO and water temperature. *A. beccarii*, *R. annectens* and *R. bradyi* were positively correlated with water temperature, trace metals and TOC content to some extent, and negatively correlated with water depth, salinity and pH. *Q. akneriana*, *P. granosum* and *A. pauciloculata* were positively correlated with food availability (Chl a concentration in the water column and chlorine content in sediments), and negatively correlated with sand content and calcium carbonate. In contrast, the species *E. adverm* exhibited an inverse pattern to the above mentioned three species.

4. Discussion

4.1. Modern benthic foraminiferal biogeography in the Pearl River Estuary

In this study, the living and dead foraminifera were observed at most of the sampling stations in the Pearl River Estuary (Fig. 3). However, samples barren of living foraminifera dominated the upper estuary, where environmental conditions are unfavorable for growth and reproduction of benthic foraminifera. Near the river mouths, there is large seasonal variability of the estuarine environments, particularly in terms of salinity variation (ranges from about 0 in summer to >15 in winter, respectively) (Dong et al., 2004). Previous culture experiments have highlighted that extreme salinity conditions promote high levels of physiological stress for benthic foraminifera, mainly through cellular osmosis (Scott et al., 2001; Murray, 2006, 2007). Below a salinity of 10–12, a cell can stop functioning unless the organism maintains a large internal ionic composition, through osmoregulation (McLusky and Elliott, 2004). This can explain partly the low densities in the upper estuary where bottom water salinity was less than 14.5 throughout the year. In addition to salinity, the absence and low abundance of foraminifera may be due to the early-diagenetic reactions that dissolve the test walls of the foraminifera under low pH (<7.8) conditions (Li, 1988) and the exposure to high-intensity human activities (e.g., trace metals and nutrient loading) (Leorri et al., 2008; Li et al., 2014; Mojtahid et al., 2016), at least to some extent. As a result, the unfavorable environments may be considered as the main stress in the upper estuary.

The abundance and species richness of living benthic foraminifera increase progressively seaward, and abundances over 100 individuals were reached in samples collected from the lower estuary (Fig. 3), which could be explained as a consequence of more stable marine conditions prevailing in the lower estuary. The most common living species found in the estuary were *Q. akneriana* and *A. beccarii*. In general, *Q. akneriana* is present in high proportions in the middle estuary, whereas *A. beccarii* has a slightly irregular distribution pattern within the estuary. For the use of benthic foraminifera as bio-indicators, the FOBIMO (Foraminiferal Bio-MOnitoring) initiative recommends a target value of 300 counted individuals (Schönfeld et al., 2012). Even though in marginal marine environments like estuaries, counts of 100 individuals are required to guarantee a reliable representative of benthic foraminiferal assemblage (Fatela and Taborda, 2002). Therefore, the amount of sample studied in this work (50 g) may not be enough to obtain a representative living benthic foraminiferal assemblage in our study region.

Similar to that of LAs, the total foraminiferal assemblage, with much higher abundance and species richness, was dominated by four species: *Q. akneriana*, *A. beccarii*, *E. advenum* and *R. annectens*. This is in agreement with previous findings in the study area (Li et al., 2014; Wu et al., 2015). These species have also been reported as dominant in other temperate estuaries and in particular the Asian estuaries (bays), such as the Changjiang Estuary, Bohai Bay, and bays of Hainan Island, China (Wang et al., 1988; Wang et al., 2016; Li et al., 2021) and the Pennar River Estuary, eastern India (Sundara Raja Reddy et al., 2009). In general, they have been suggested as the most euryhaline among the hyaline species and therefore can tolerate large salinity variations. For example, *A. beccarii* is commonly recognized in transitional environments under pollution stress and is tolerant to a variety of environmental conditions (Murray, 2006), whereas *Q. akneriana* shows a preference for intermediate salinities and oxygen-enriched environment (Garrett, 2010; Platon et al., 2005).

Generally, living foraminiferal assemblages recorded based on the time of sampling, whereas dead assemblages are built up over a period of time (~1–2 years in our study) and therefore reflect the cumulative effects of annual production and in some cases transport of foraminifera (Murray, 2006; Leorri et al., 2008; Li et al., 2021). Compared with the living counterpart, the relatively more abundant and diversity of total assemblages (live plus dead) (10 and 6 times higher on average) could be a result of (1) the occurrence of allochthonous taxa transported by tidal currents from the shelf, and/or (2) seasonal differences of living assemblages due to the impact of seasonally varying physical chemical parameters.

The prerequisite of foraminiferal proxies is that the foraminiferal assemblages should be predominantly found in situ (Sejrup et al., 2004; Leorri et al., 2008). Based on three lines of evidence, however, we argue that the total foraminifers in surface sediment of the Pearl River Estuary were predominantly autochthonous. Firstly, the total benthic foraminifera in the estuary have similar dominant species as in the living assemblages (but the rank orders differ slightly). Secondly, the shells of dead foraminifera in most of the surface sediment samples were basically preserved intact and varied broadly in size. In contrast, the exotic species transported by tidal currents is made up of small, thin walled forms or abraded empty shells (Cearreta, 1988). Thirdly, the PRE is a typical weak tidal influenced estuary with a mean tidal range between 1.0 and 1.7 m, and the redistribution of foraminifera or transport of exotic species by tidal currents is expected to be minimal (Huang and Yim, 1998; Wu et al., 2015), although the transport of allochthonous species can be important to the total foraminifera in certain regions, notably in the deep channels (Luo et al., 2001) which has been precluded during our sampling. Thus, it is reasonable to speculate that the total faunal composition is representative of the population dynamics of benthic foraminifera throughout the year.

Actually, possibly more important for the explanation for the observed dissimilarity between living and total foraminiferal assemblage is the seasonal variations in living assemblage structure. The study area is under the strong influence of seasonal monsoon. High discharge commonly occurs in summer, when recorded salinity values were at a minimum (Zhao, 1990; Harrison et al., 2008). Moreover, increased freshwater influx was also reflected in the overall water quality, when high input of nutrients together with high concentrations of trace metals was observed (e.g., Dai et al., 2008; Zhang et al., 2013). These stressors can together lead to the low number and low diversity of foraminifera. In contrast, stable hydrographic conditions exist in the winter season, which could be favorable for the growth of living benthic foraminifera. There are other reports on similar cases, such as the Chilika lagoon, India and Hamble Estuary, England (Alve and Murray, 2001; Jayalakshmy and Rao, 2001; Sen and Bhadury, 2016). For example, the main reproductive periods are pre-monsoon and/or post-monsoon months for the most dominant taxa of living assemblage (including the genus of *Ammonia*) in Chilika lagoon, whereas the monsoon season is characterized by the lowest abundance and lowest diversity (Sen and Bhadury, 2016). Therefore, the seasonal lowering of salinity together with increased pollutant loading may have accounted for the low richness and density values of living foraminifera in the Pearl River Estuary. Clearly, further work on the seasonal dynamics of living assemblages and its link to the physio-chemical parameters is required to confirm our hypothesis.

4.2. Environmental factors controlling benthic foraminiferal assemblages

The temporal dynamics and spatial distribution of recent benthic foraminifera in marginal environments are controlled by the variable environmental conditions such as salinity, deposition rates, and organic matter flux. Notably, the leading roles of hydrodynamics, food availability and sedimentary features on benthic foraminiferal assemblages in estuaries have been documented in many field studies (Nikulina et al., 2008; Mojtahid et al., 2009, 2016; Wang et al., 2016). In the PRE, previous studies have mainly focused on the role of hydrodynamics and sediment features on the distribution and ecology of foraminifera (Li, 1985; Garrett, 2010; Wu et al., 2015). In the present study, the multivariate analysis shows that the density and species richness of total foraminifera are positively correlated with salinity and sand content (Table 1), further supporting the previous argument that the hydrodynamic conditions and sediment type are major factors controlling benthic foraminiferal assemblage in the region (e.g., Luo et al., 2001; Li et al., 2015; Wu et al., 2015). Moreover, according to the cluster analysis, three main clusters for the total 30 sampling stations were identified (Fig. 5). These assemblages seem to be associated with the distinct hydro-sedimentary conditions, that is, from a hyposaline influence assemblage in upper and middle estuary, hyposaline to moderate marine in the lower estuary.

In addition to the hydro-sedimentary control, a number of other stress factors also disturb the living foraminifera and cause significant spatial variability of benthic micro-faunal assemblages. For example, food availability plays an important role in controlling foraminiferal communities in transitional environments (Gustafsson and Nordberg, 2000). According to correlation analysis (Table 1, Fig. 6), the high proportions of *Q. akneriana* are associated with high amounts of *Chl a* in the water column. This may indicate that the availability of freshly phytoplankton is a major controlling factor for the species *Q. akneriana*. Trace metals and organic pollutants are widely regarded as major structuring forcing that have an adverse effect on the benthic faunas

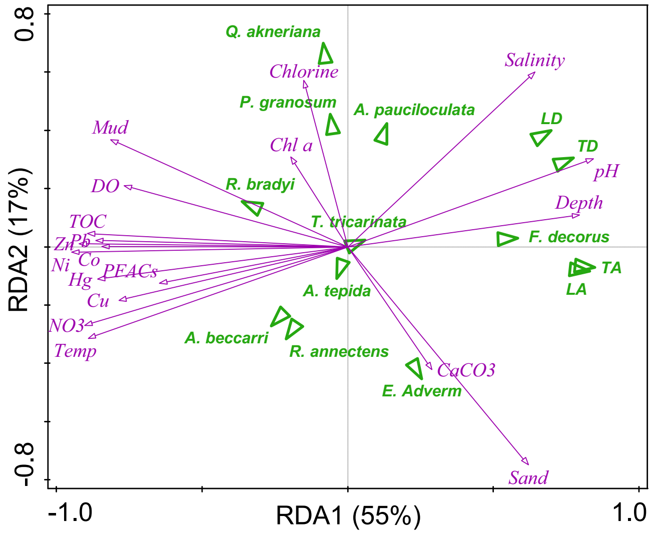

Fig. 6. RDA ordination diagram of forminiferal assemblage/taxon associated with environmental variables.

(Nikulina et al., 2008; Munsel et al., 2010; Martins et al., 2016; Li et al., 2021). However, in our case study, the multivariate analyses reveal that trace metal pollution (particular Cu) appears as an important factor on the spatial distributions of *A. beccarii* and *R. annectens* (Table 1, Fig. 6). It is noteworthy that trace metals are preferentially adsorbed onto the fine-grained organically rich sediments, therefore making it difficult to separate the effects of pollutants on benthic foraminifera from that of fine grained sediments. Nevertheless, the weak relationship between *A. beccarii* and the sand or silt/clay fraction (Table 1) support the idea that the trace metal influences override the sand effects.

In general, trace metals have the ability to enter into the cell of benthic foraminifera with food and become toxic to this fauna (Yanko et al., 1998; Le Cadre and Debenay, 2006). Reduced growth rates may be a generalized response of foraminifera when exposed to toxic trace metals. Each species has its own threshold of sensitivity to different types and degrees of pollution, over that the toxic effects can induce changes in faunal composition. Among these species, *A. beccarii* has been considered as a resistant species to certain trace metals (notably Cu in this study) and high organic matter while *Q. akneriana* is recognized by its intolerant to pollution by trace metals and low oxygen environments (Thomas et al., 2000; Tsujimoto et al., 2006; Mojtahid et al., 2009). In fact, laboratory-based experimental studies have shown that the genus *Ammonia* are very sensitive on one hand, but also particular resistance to Cu enrichment on the other hand, with the lethal value can be as high as 200 to 400 μg/L in seawater (Sharifi et al., 1991; Le Cadre and Debenay, 2006). On the basis of the results of X-ray analyses, Samir and El-Din (2001) suggest that Cu, followed by Zn, is more easily absorbed by *A. beccarii* than other elements such as Pb and Ni. Furthermore, the research of Li et al. (2013) indicates that *A. beccarii* (referred as *A. tepida* in the study) is absent from the foraminiferal assemblage outside the PRE when the Cu concentration is less than 20 mg/kg in sediments. We note that the region outside the PRE where *Ammonia* is absent is far beyond the spatial coverage of samplings in the current study. However, based the above mentioned evidence, our findings confirm the capacity of the *Ammonia beccarii* to tolerant increasing trace metal pollution, particularly the Cu contamination. The extent to which such abiotic processes may influence the foraminiferal assemblages warrants further study.

As a whole, our results suggest that the distribution patterns of benthic foraminifera in the Pearl River Estuary are largely controlled by hydrodynamic conditions and sediment features. In addition, we also highlight bottom water phytoplankton biomass and trace elements as important force driving assemblage composition, particularly the species of *A. beccarii* and *Q. akneriana*.

4.3. Temporal variability of foraminiferal assemblages: a comparison with previous results

How do benthic foraminiferal assemblages during 2011 compare to similar faunas data based on samples collected earlier (>1985, 1988, 2004, 2006/2009 and 2010) in the Pearl River Estuary? Table 2 presents the total foraminiferal faunal characteristics observed in different studies over the last 30 years, when the human intervention was manifested. Note that the locations of sampling stations were slightly different, and thereafter we will only consider stations/areas from 2011 situated in previous reports (Fig. 1). The results show that the variations in density and species richness of foraminifera were small between 1980s and 2010s in both upper and middle estuary, despite that the environmental conditions in these areas have been degraded over the last 30 years. Regarding the lower estuary, we can document an overall decreasing trend in density and species richness over the past 3 decades (Fig. 7). Furthermore, there were distinctly different foraminiferal assemblages in the last decades: some species (e.g., *Miliolinella* sp. and *Brizalina striatula*) have apparently disappeared or decreased in 2000s, while the relative abundances of *A. beccarri/A. tepida* and *Q. akneriana* are more abundant and widespread in 2000s as compared to 1980s, particularly in the middle and lower estuary (Table 2). For example, *A. beccarri/A. tepida*, a stress tolerant species commonly present in the coastal and paralic environments, was not common in the middle-to-lower estuary in 1980s, but it presented as the dominant species in 2010s.

Previous works have documented that the recent benthic foraminiferal assemblage in surface sediments of the PRE is mainly controlled by water salinity and sediment type (Garrett, 2010; Wu et al., 2015; Chen et al., 2019). In the present study, the population dynamics of foraminiferal assemblage also present a positive correlation with salinity (Table 1). Over the last 3 decades, however, there was no obvious long-term change in freshwater input from the Pearl River, whereas the sediment influx was decreasing dramatically, primarily due to the construction of more than 8600 reservoirs from the late 1990s (Zhang et al., 2008; Wu et al., 2016). As a consequence, salinity remained constant throughout the whole estuary, but the sand content of surface sediments exhibited a significant decrease, notably in upper estuary (Jia et al., 2011; Yuan et al., 2019). In contrast, there was no substantially change in density and diversity of the foraminiferal assemblage in both upper and middle estuary. Moreover, the reduction of sand content alone does not cause the shift in dominant species composition as well as the increased proportion of *A. beccarii*, since there are no significant relationship between sand content and these species (Table 1). Thus, the hydrodynamic conditions and sediment features, even though have a strong influence on population structure of benthic foraminifera, are unlikely to be the most important factor for the foraminiferal assemblage change in the lower estuary during the last 3 decades.

From another point of view, we propose that the elevated metal contamination and increased food availability due to strong human perturbation are responsible for these changes. There are mainly three reasons. First, *A. beccarii* showed a close relationship with Cu and other

Table 2
Summary of the dominant species of benthic foraminifera in the literature and in this study of the Pearl River Estuary.

Year	Upper estuary	Middle estuary	Lower estuary	References
before 1985	*Ammonia beccarii* *Elphidium nakanokawaense*	*Rotalidium annectens* (*Ammonia annectens*[a]) *Brizalina striatula*	*Elphidium advenum* *Hanzawaia nipponica*	Li, 1985
1988	*Ammonia beccarii* *Elphidium nakanokawaense*	*Ammobucalites formosens* *Haplophrugmoides canariensis*	*Miliolinella* spp. *Quinqueloculina akneriana*	Luo et al., 2001
2004	*Ammonia tepida* (*Ammonia beccarri*[a])*-Haplophragmoides* *Canariensis-Elphidium nakanokawaense*		*Ammonia* spp. *Elphidium advenum*	Li et al., 2014
2006/2009	*Ammonia baccarii*	*Ammonia baccarii* *Haplophragmoides* spp.	*Ammonia baccarii* *Elphidium advenum*	Garrett, 2010
2010	*Ammonia tepida* (*Ammonia beccarri*[a]) *Elphidium excavatum*	*Ammobaculities formosensis* *Rotalidium annectens* (*Cavarotilia annectens*[a])	*Ammonia tepida* (*Ammonia beccarri*[a]) *Elphidium advenum*	Wu et al., 2015
2011	*Ammonia beccarii* *Rotalidium annectens*	*Quinqueloculina akneriana* *Ammonia beccarii*	*Ammonia* spp. *Hanzawaia nipponica*	This study

[a] *Rotalidium annectens/Ammonia beccarii* identified in our study was reported as *Ammonia annectens* and *Cavarotalia annectens/Ammonia tepida* in previous researches (Li, 1985; Huang and Yim, 1998; Luo et al., 2001; Li et al., 2014; Wu et al., 2015).

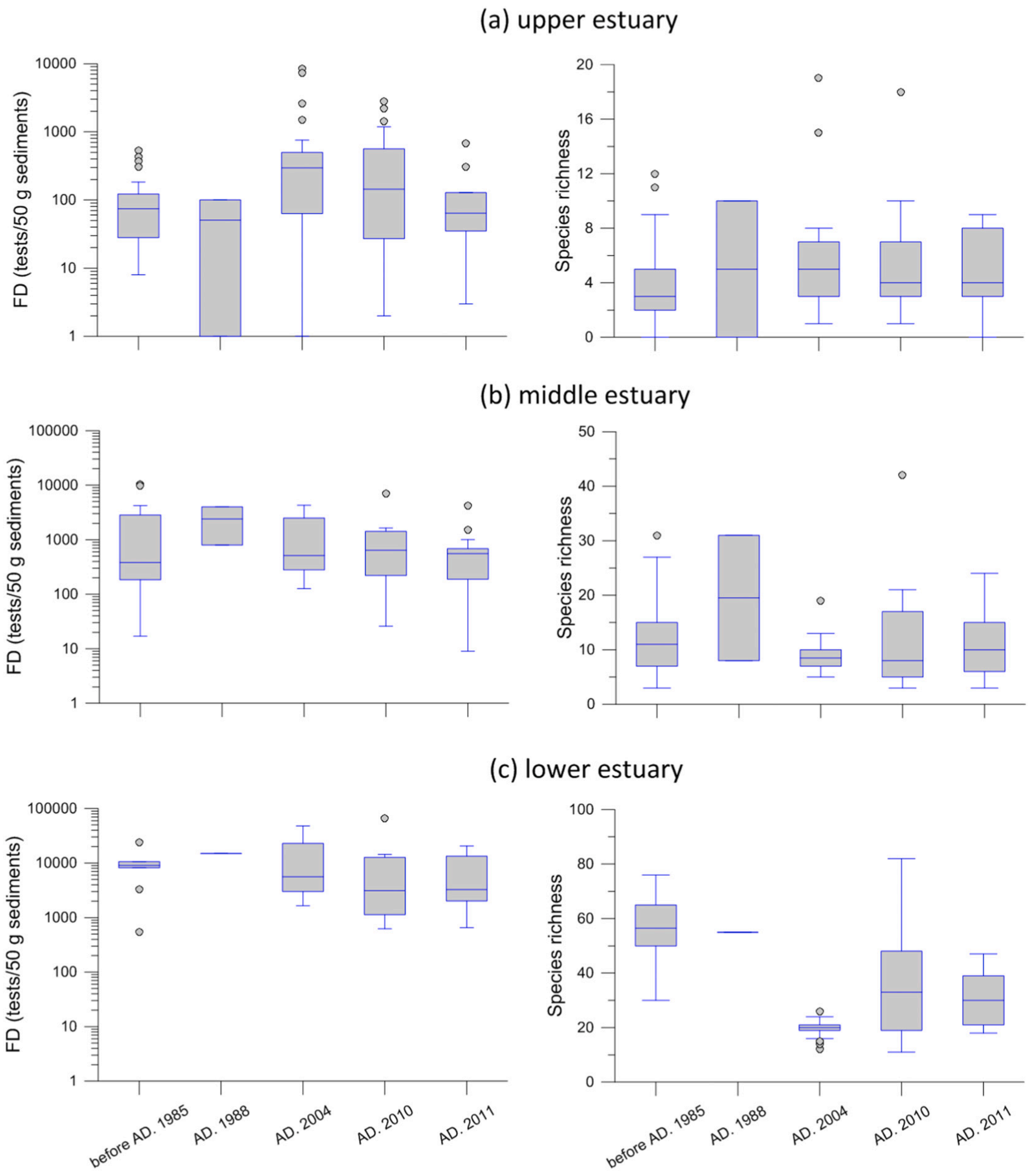

Fig. 7. A comparison of the foraminiferal density (left panels) and species richness (right panels) from different sub-regions of the Pearl River Estuary during the last three decades.

Table 3
Summary of trace metal (unit: mg/kg) and total organic carbon (unit: %) data in the literature in surface sediments of the PRE.

Sampling Year	Ni	Zn	Cu	Pb	Hg	TOC	Reference
1980		80–100	20–40	30–40	0.20–0.30		Tang, 1983
1987–1988		126	37	58		0.92–1.0 (19)	Cai and Han, 1990
1997		110.9 (n = 21)	39.0 (n = 21)	59.4 (n = 21)	0.35 (n = 21)		Liu et al., 2003
2004	43.9 (n = 55)	179.8 (n = 55)	55 (n = 55)	59 (n = 55)	0.17 (n = 66)	1.06 (n = 55)	Shi et al., 2007; Li et al., 2014
2008	36.7 ± 6.0 (n = 23)	176.8 ± 43.4 (n = 23)	45.7 ± 15.4 (n = 23)	57.9 ± 11.9 (n = 23)			Yu et al., 2010
2010		150.0 (n = 21)	48.5 (n = 21)	51.0 (n = 21)	0.20 (n = 16)	1.19 (n = 16)	Wang et al., 2012; Yu et al., 2012
2011	33.7 ± 9.3 (n = 39)	139 ± 40.9 (n = 39)	54.6 ± 32.3 (n = 39)	36.1 ± 9.7 (n = 39)	0.09 ± 0.03 (n = 39)	1.57 (n = 39)	Chen et al., 2012; Yin et al., 2015

trace metals (Ni, Pb, Zn and Hg), especially in the polluted areas, while *Q. akneriana* is more linked to phytoplankton biomass and oxygen content, as discussed above (Table 1, Fig. 6). Second, the phytoplankton biomass, organic carbon and severity of Cu contamination in surface sediments (and in dated sediment cores) appeared to be increasing over the last decades (Table 3), although the effective reduction on pollution sources has been implemented in recent years (e.g., Chen et al., 2012; Duan et al., 2014). Last but not least, the less variable and low diversity in the more polluted upper and middle estuary may indicate that the foraminiferal assemblages have been influenced predominantly by terrestrial materials in these areas, and is thus insensitive to its flux over the past decades. Thus, the benthic foraminiferal assemblage could be potentially used to trace changes in pollution stress for the past. We note that the long-term trends should be considered with caution due to the limited sample years, but it still confident that the foraminifera are undergoing an alarming decreasing trend in species densities and species richness in the lower Pearl River Estuary.

5. Conclusions

The modern benthic foraminiferal data from the Pearl River Estuary show a clear link with environmental parameters. Species richness and density of living faunas are low in the upper and middle estuary, primarily due to the unfavorable environmental conditions including deposition rates and hydrodynamic conditions prevailing in this area. For the total foraminifera, the spatial distribution and population dynamics were mainly controlled by hydro-sedimentary characteristics. On the other hand, the availability of freshly phytoplankton and trace metal pollution, particularly Cu, may have played an important role on the faunal composition. The comparison of faunal data with previous investigations shows that the foraminiferal assemblage has dramatically changed over the last three decades. We inferred that the increased intensity of anthropogenic perturbations (notably Cu contamination and eutrophication) may contribute to the reductions in foraminiferal abundance and diversity in the lower estuary. Further laboratory and in situ studies are needed to better understand the ecological effects of trace metal and eutrophication on living benthic foraminiferal assemblages in highly polluted coastal and estuarine systems.

CRediT authorship contribution statement

Feng Ye: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Xiaoping Huang:** Conceptualization, Writing – review & editing. **Zhen Shi:** Conceptualization, Writing – review & editing. **Baowei Chen:** Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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