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Full Length Research Paper

Variation of abundance of *Planctomycetes* in typical aquatic environments of the China seas

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The CARD-FISH approach with the HRP labeled oligonucleotide probe Pla-46 was applied to investigate the abundance of *Planctomycetes* in the China seas. Our data subtly revealed that the abundance of *Planctomycetes* was varied from 0.23 to 20.53×10^4 cells/ml in 10 sampling sites of Yangtze River estuary and from 0.15 to 10.25×10^3 cells/ml in the 3 water depth profiles of the South China Sea (the maximum abundance found in the euphotic zones) respectively. Even though 20 times higher than in the water depth profiles of the oligotrophic South China Sea, the abundance of *Planctomycetes* in Yangtze River estuary is lower than that of in soils and sediments according to previous publications. To the best of our knowledge, the present work is the first systematic assessment and comparison of the abundance of *Planctomycetes* in typical aquatic environments. Our results showed that the variation of abundance of *Planctomycetes* differed with hydrological, physical, and chemical features, providing the patterns of the abundance in oligotrophic water, estuary, and water depth profiles; this is important information of their quantitative distribution, potential ecological roles.

Key words: *Planctomycetes*, the China Seas, CARD-FISH, abundance, seawater.

INTRODUCTION

Planctomycetes is characteristics with its peptidoglycan-less cell wall, budding reproduction and unique cell organization features which make it a model micro-organism to understand the evolutionary relationship between the prokaryotes and eukaryotes (Konig et al., 1984; Fuerst, 1995). These bacteria have been identified in various environments such as freshwater (Elshahed et al., 2007), marine water column (Kirkpatrick et al., 2006), marine sediments (Musat et al., 2006), soil habitats (Buckley et al., 2006), and municipal wastewater treatment plant (Chouari et al., 2003). So far, *Planctomyces*, *Pirellula*, *Gemmata*, *Isosphaera*, *Schlesneria, Singulisphaera* and anaerobic *Planctomycetes* performing anaerobic ammonia oxidation (anammox) have been validly described (Krieg and Garrity, 2008). As a significant member of the bacteria domain, the heterotrophic and chemoautotrophic *Planctomycetes* exhibited an increasing significance for microbial ecology because of ubiquitous distribution, important biogeochemical and potential ecological metabolism in marine environments, such as anammox proccess which might contribute to the major nitrogen loss term in oxygen minimum zones of the oceans (Codispoti et al., 2001; Elshahed et al., 2007).

The application of uncultured independent approaches including clone library (Kulichevskaya et al., 2006) and fluorescent in situ hybridization (fish) techniques (Ishii et al., 2004; Gade et al., 2004), have revealed the ubiquitous distribution of *Planctomycetes* as mentioned earlier. Previously, many publications have reported that the *Planctomycetes* was the most numerous bacterial group in many polluted habitants, such as a quantitative study in a pristine forest soil from Switzerland (Zarda et al., 1997), a fish study of seasonal changes in two polluted rivers (Brummer et al., 2004) and recent study with high abundance in anoxic layers of a sphagnum peat bog and surfaces of the kelp *Laminaria hyperborea*

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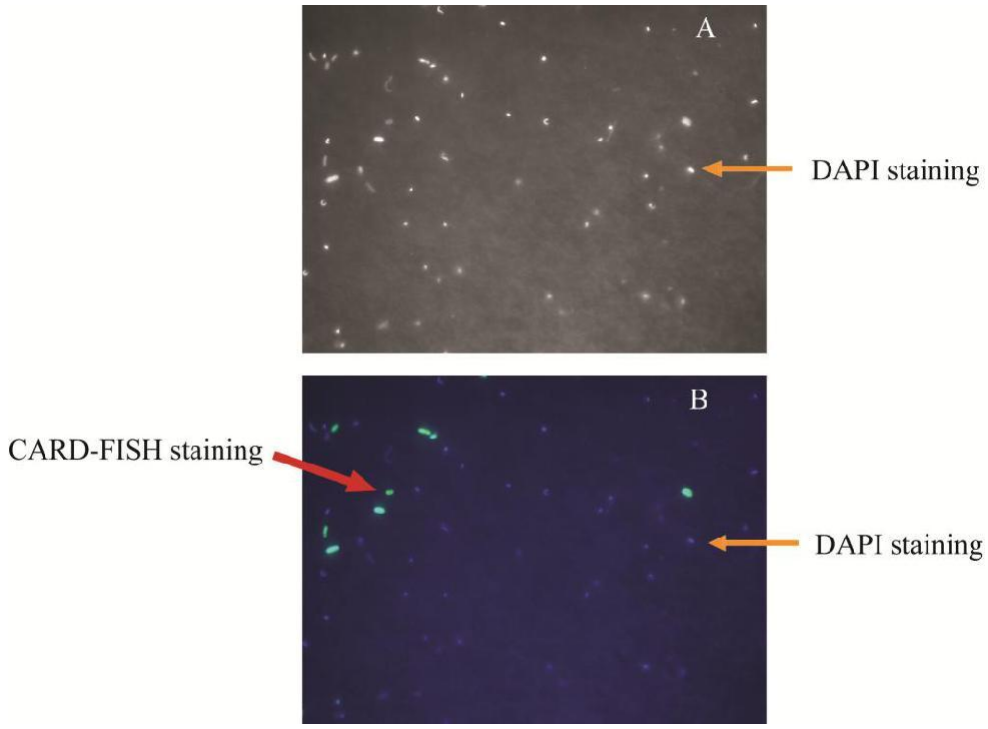

Figure 1. Images of CARD-FISH and DAPI staining.

(Ivanova and Dedysh, 2006; Bengtsson and Ovreas, 2010). Although the abundance might lead to microbial ecological role of the *Planctomycetes* in marine environments, the past few years have nonetheless seen a flood of papers reporting the quantitative study of *Planctomycetes* in marine water ecosystems. Since most *Planctomycetes* in aquatic habitats are small, slow growing, or starving, and the signal intensities of hybridized bacterioplankton cells were frequently below the detection limits or lost in high background fluorescence (Fuerst, 1995; Morita, 1997), the recently developed catalyzed reporter deposition fish (CARD-FISH) method provide more sensitive investigation of *Planctomycetes* in contrast of FISH technique, with the use of oligonucleotide probe labeled with horseradish peroxidase (HRP) (Pernthaler et al., 2002).

As for *Planctomycetes* abundances in typical environments, such as oligotrophic water, estuary, and water depth profiles, the data is still scarce. Since poor aquatic data, we purposively investigated the water depth profiles of the South China Sea (marginal seas of the Northwest Pacific characterized with changeable water pressure, salinity and oligotrophic) and Yangtze River estuary (characterized by large amounts fresh water and nutrient input) (Hu et al., 2011), which represent oligotrophic water and estuary environments respectively, to reveal the abundance pattern of aquatic *Planctomycetes*. Furthermore, many publications reported that the high abundance of such bacterial group was usually involved in polluted habitants and algae bloom, indicating some *Planctomycetes* species closely related to aquatic pollution (Fuerst, 1995; Morris et al., 2006). Thus, the investigation of abundance of *Planctomycetes* in typical aquatic environments was meaningful for the evaluation of its ecological role and its relationship related to different water bodies. In this paper, we applied CARD-FISH method to determine the number of *Planctomycetes* in such typical aquatic environments of the China seas, with deeper understanding of the quantitative distribution, their potential ecological roles.

MATERIALS AND METHODS

Samples

A fan-shape transaction from the Yangtze River estuary to the open water of Yangtze River estuary was sampled during a summer cruise (September, 2006) (Figure 1). Total and 2-20 μm size-fraction chlorophyll were measured by the acetone extraction fluorescence method (Holm-Hansen et al., 1965) using 250 ml of seawater samples. Characteristics of the investigated sampling sites of Yangtze River estuary is given in Table 1. Three water depth profiles of the South China Sea including the total of 19 water layers, at sampling sites Z97 (118.97497 E, 17.9594 N, water depth: 4130 m) Y32 (110.1711 E, 13.4523 N, water depth:2390 m) and S2 (115.91962 E,18.784133 N, water depth: 3300 m) (Figure 1), were sampled during a winter cruise (November, 2006). The basic characteristics of the water depth profiles, such as temperature and salinity, are given in Figure 3. All samples of 20 ml were immediately fixed with fresh paraformaldehyde (2% final

Table 1. Environmental parameters for all sampling stations of the East China Sea.

Sampling sites	Longitude (°)	Latitude (°)	Temperature (℃)	Salinity	Total Chlorophyll (μg/L)	2-20 μm size-fraction Chlorophyll (μg/L)
CJ1	122.014	32.229	27.8	25.08	1.2807	0.4925
CJ2	122.5	32	26.5	29.55	0.43224	0.1465
CJ3	123	32	25.2	31.2	2.1931	1.3881
CJ4	123.5	32	25.6	31.2	1.3108	0.5482
CJ5	123.5	31.5	26.3	31.2	0.8526	0.4345
CJ8	122.2	31.5	26.5	20.3	0.7194	0.308
CJ9	122.3	31.2	27.2	22.5	1.0281	0.4402
CJ11	122.6	31	27.1	28.1	2.285	1.5997
CJ13	123	30.8	26.7	30.1	3.8886	1.4667
CJ14	123.5	30.5	28	32.3	1.1467	0.3465

concentration) and stored at 4°C for 15 to 30 min, then the samples were filtered onto 0.2 μm pore size white polycarbonate membrane filters, supported with 0.45 μm nitrocellulose membranes (Whatman), washed with deionized water and stored at –20°C until analysis (Jiao and Zhang, 2006).

CARD-FISH procedure

The filters were dipped with both sides into the low gelling point agarose and air-dried on a paper tissue, then permeabilized with fresh lysozyme solution (10 mg/ml) in lysozymebuffer (0.05 M EDTA, pH 8.0; 0.1 M Tris HCl, pH 8.0). The cut filters in sections were hybridized in the mixture of hybridization buffer and probe (300:1). After washing and drying, the sections were amplified in the 1000 μl amplification buffer with 10 μl of the 100 ×H_2O_2 stock and 2 μl of fluorescently labeled tyramide. The filter sections were counterstained with the DNA stain 4´, 6´-diamidino-2-phenylindol (DAPI), and finally were performed to microscopic analysis after embedding.

Horseradish peroxidase (HRP) -labeled probe

The HRP-labeled oligonucleotide probe PLA46 (5'-GACTTGCATGCCTAATCC-3'; targeting *Planctomycetes*) is applied in this study, which has been successfully applied in previous related studies (Gade et al., 2004; Neef et al., 1998; Musat et al., 2006). In hybridization buffer, the final formamide concentration of probe PLA46 is 30%.

Microscopic analysis

For each slide, about random 20 fields are accounted for DAPI-stained or probe-combined cells. The total number of cells per milliliter are accounted by the formula: *n*cell/ ml = *A* ×*S1*/ (*S2* ×*V*); where *A* is average numbers of 10 fields; where *S1* is a field area under microscopy and where *S2* is useful area of a filter and *V* is the volume of sampling water.

RESULTS AND DISCUSSION

To the best of our knowledge, the present work is the first systematic assessment of the abundance of *Planctomycetes* in typical aquatic environments with different hydrological features. Our data profiled the abundance patterns of *Planctomycetes* in the oligotrophic South China Sea and Yangtze River estuary, and presented the abundance variation and comparison among the investigated samples.

Abundance of Planctomycetes in the water depth profiles of the South China Sea

Three water depth profiles including the total 19 water samples were investigated using the *Planctomycetes* specific probe PLA46 labeled HRP (Figure 1). Our results showed that abundance of *Planctomycetes* varied from 0.32 to 9.98×10^3, from 0.15 to 1.29×10^3, and from 0.53 to10.25×10^3 cells/ml, corresponding to from 1.93 to 11.23‰, from 0.85 to 9.29‰ and from 0.53 to 10.25‰ of the proportion of the total DAPI counts, in the water depth profiles Z97, Y32 and S2 respectively (Figure 3). With the increasing water depths and decreasing water temperature, the abundance of *Planctomycetes* exhibited obvious changes in surveyed depth profiles of the South China Sea, distributing from 0.15 to 10.25×10^3 cells/ml in cells numbers and occupying from 0.53 to11.23‰ of the proportion to the total DAPI counts. It seemed that the maximum abundance of *Planctomycetes* related to the euphotic zones from the investigations of the water depth profiles S2 and Z97, potentially since occupying high abundant of light-depend genus such as *Pirellula*,

Blastopirellula, and *Rhodopirellula* according to previous publication (Shu and Jiao, 2008). The highest abundance difference between at the 75 m depth layer of the profile S2 and at 5 m depth layer of the profile Z97 possibly dues to an existence of a mini coastal upwelling occurred from 25 to 150 m of the site Z97, which may resulted in replete nutrient and unstable stratification. For the deep water layers, the layer close to seafloor of the depth profile Y32 (the water depth: 2390 m) showed higher abundance of *Planctomycetes* than in the approximate layers of the depth profiles S2 (water depth: 3300 m) and Z97 (water depth: 4130 m) because of the exchange between the water column and the sediment by perturb-bation (Figure 3). Our results indicated the abundance and distribution of *Planctomycetes* in the water depth profiles of the South China Sea were possibly related to euphotic zones, water temperature and the exchanges of organic matters.

Abundance of *Planctomycetes* in the Yangtze River estuary

Ten summer samples from the East China Sea near to the Yangtze River estuary were analyzed using *Planctomycetes* specific probe PLA46 labeled HRP. By accounting, the DAPI counts varied from the maximum of 3.76×10^6 (at site CJ1) to the minimum of 1.97×10^7 cells/ml (at site CJ5). HRP-labeling PLA-46 probe revealed the maximum of *Planctomycetes* cells of 2.05×10^5 cells/ml at site CJ5 and the minimum of 0.23×10^4 cell/ml at site CJ14 while the highest percentage of 1.58% at site CJ4 and the lowest percentage of 0.44% to the total DAPI counts at site CJ9 respectively.

From Figure 2, the *Planctomycetes* of the sites along the Yangtze River estuary including sites CJ8, CJ9, CJ11, CJ13, and CJ14, exhibited lower abundance (0.23 to 1.68×10^4 cell/ml) than that of the sites upstream including sites CJ1, CJ2, CJ3, CJ4, and CJ5 (3.45×10^4 to 2.05×10^5 cell/ml) (Figure 2), this possibly owed to the large input of freshwater of the Yangtze River with high organic matters in summer (Tadonleke, 2007). Additionally, the statistical analysis showed that water salinity has positive correla-tion (R^2 = 0.453) and water temperature has negative correlation (R^2=0.60913) to the abundance of *Planctomycetes* respectively. Interestingly, unlike pre-vious reports (Morris et al., 2006), there was no closely relationship between high abundance of *Planctomycetes* and algae bloom in the sampling sites CJ3, CJ11 and CJ13.

Contrast of abundance of *Planctomycetes* in marine environments

In our ecological investigation, the culture-independent approach CARD-FISH techniques with *Planctomycetes* specific probe presented the variation of abundance in the surface of Yangtze River estuary and in the water depth profiles of the South China Sea, providing the basic quantitative information of *Planctomycetes* in various seawater environments. Taken all investigated sites together, it is interesting that the abundance or percentage of *Planctomycetes* to the total DAPI counts in Yangtze River exhibited average 20 times higher than that in oligotrophic water depth profiles of the South China Sea. The reasons are: 1) the study sites in Yangtze River estuary are greatly influenced under the terrestrial input of nutrient which may be mainly response to the variation of such widespread bacterial group, such as the content of organic carbon which was considered as a key factor to distribution of *Planctomycetes* (Tadonleke, 2007); 2) there is a seasonal changes of abundance of *Planctomycetes* according previous report, with sampling from Yangtze River estuary in summer while from the South China Sea in winter respectively.

Previous abundance investigations showed *Planctomycetes* a significant proportion of microbial community in various marine environments, occupying from 2 to 10% of the DAPI counts in two polluted rivers and from 4 to 13% in anoxic layers of a sphagnum peat bog (Ivanova and Dedysh, 2006) respectively. However, our results reveal lower proportion of *Planctomycetes* than previous reports with from 0.44 to 1.58% of the DAPI counts in Yangtze River estuary surface seawater and from 0.09 to 1.1% in the water depth profiles of the South China Sea, showing unique seawater *Planctomycetes* abundance and distribution.

Conclusion

In conclusion, our data revealed that the abundance of *Planctomycetes* in Yangtze River estuary (from 0.23 to 20.53×10^4 cells/ml) and in 3 water depth profiles of the South China Sea (from 0.15 to 10.25×10^3 cells/ml) respectively using CARD-FISH approach, showing the abundance of *Planctomycetes* differed with hydrological, physical, and chemical features. As for the water depth profiles, the maximum abundance of *Planctomycetes* of the euphotic zones possibly related to light-depend genus (such as *Pirellula*, *Blastopirellula*, and *Rhodopirellula*). Considering versatile ecological player in aquatic environ-ment, such as anammox process (Krieg and Garrity, 2008), C1 utilization (Kalyuzhnaya et al., 2005) and potential sulfates metabolisms (Elshahed et al., 2007), our abundance information is important to assess their potential ecological role.

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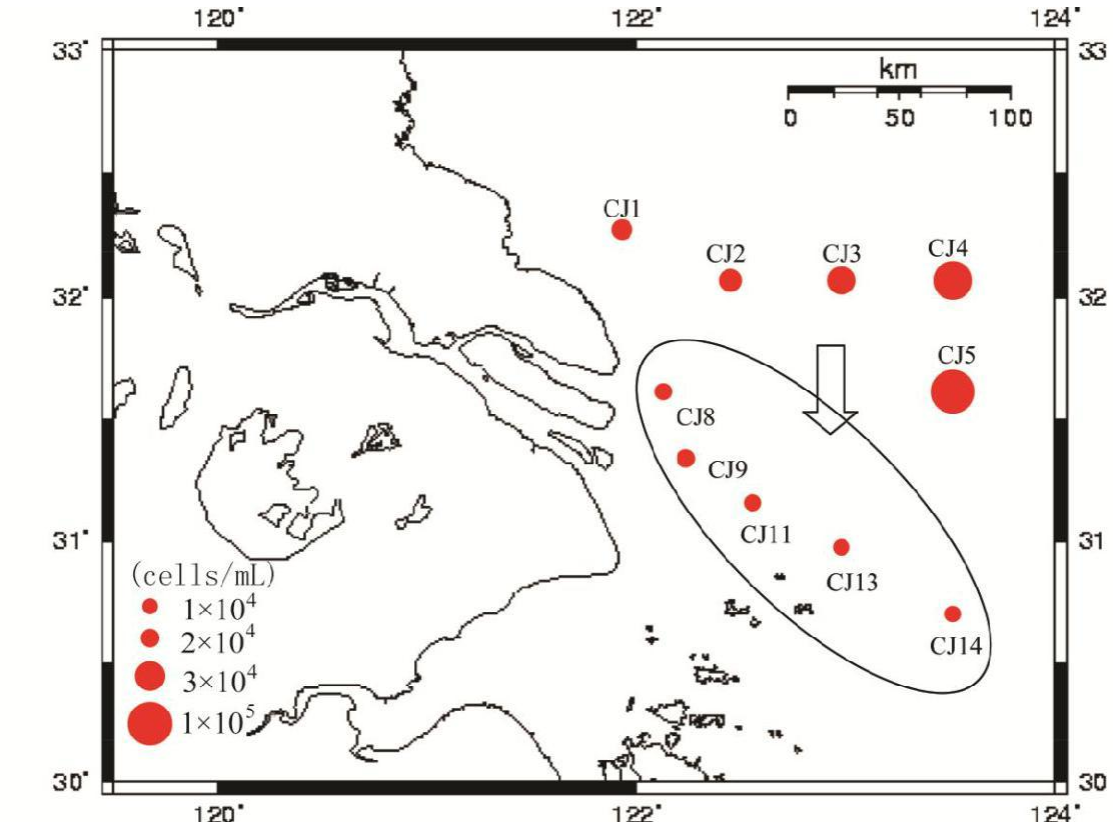
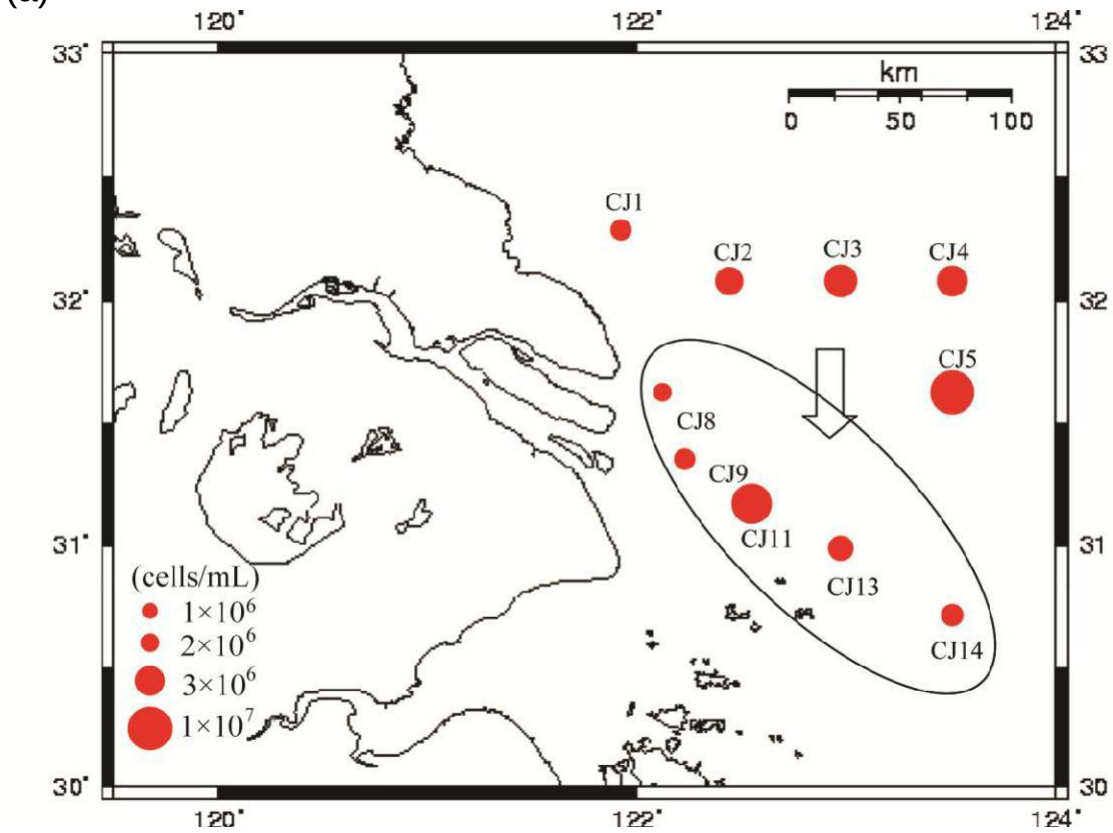


(a)



(b)

Figure 2. Abundance of *Planctomycetes* (a) and DAPI counts of the East China Sea (b).

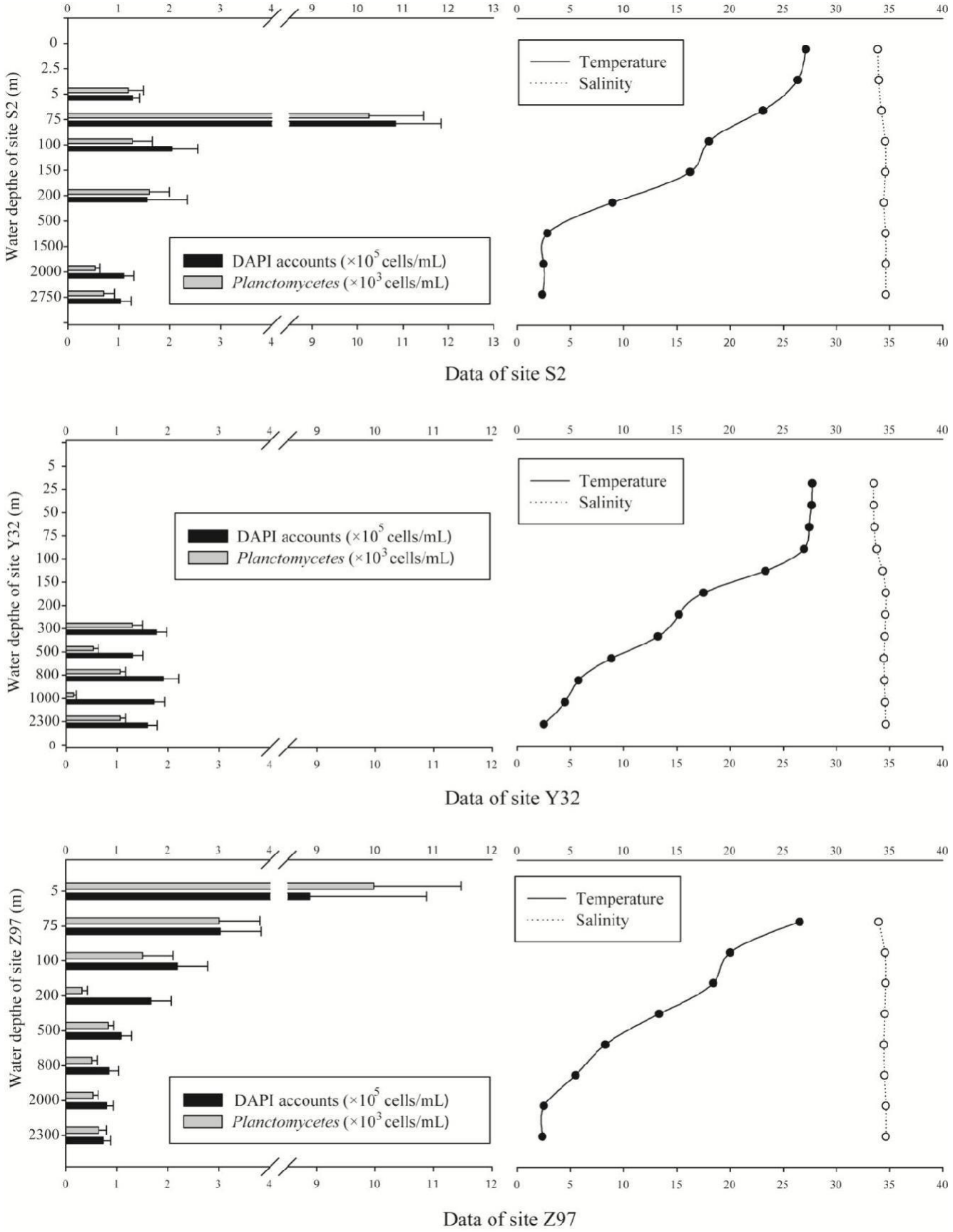


Figure 3. Abundance of *Planctomycetes* and DAPI counts in the 3 water depth profiles of the South China Sea.

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