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Size structure of biomass and primary production of phytoplankton: environmental impact analysis in the Dongsha natural gas hydrate zone, northern South China Sea

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Abstract

The size-fractionated biomass and primary production of phytoplankton, and the influence of environmental factors on it were studied in the Dongsha natural gas hydrate zone of the northern South China Sea in May 2013. Low nutrient, low chlorophyll *a* (Chl *a*) and primary productivity characteristics were found in these waters. The phenomena of subsurface Chl *a* maximum layers (SCMLs) and primary production maximum layers (SPMLs) were observed in the Dongsha waters. There were significant differences in the size-fractionated biomass and primary production that showed picophytoplankton>nanophytoplankton>microphytoplankton in terms of biomass and degree of contribution to production. Vertical biomass distribution indicated there were considerable differences among different phytoplankton within the euphotic zone (Zeu) in spring. For example, microphytoplankton was distributed evenly in the euphotic layer and nanophytoplankton was mainly distributed in the subsurface or in the middle of the euphotic layer, while picophytoplankton was mainly distributed in the middle or bottom of the euphotic layer. Smaller cell size and larger relative surface area allow picophytoplankton to benefit from nutrient competition and to hold a dominant position in the tropical oligotrophic waters of low latitudes. There was a positive correlation between size-fractionated biomass and temperature with pH and a negative correlation between size-fractionated biomass and silicate with phosphate. There was a positive correlation between size-fractionated primary production and temperature and a negative correlation between size-fractionated biomass and salinity with phosphate. Phosphate was an important factor influencing the size structure of phytoplankton. Meanwhile, irradiation and the euphotic layer were more important in regulating the vertical distribution of size-fractionated phytoplankton in the Dongsha natural gas hydrate zone.

Key words: phytoplankton, biomass, primary production, size fractionation, gas hydrate, northern South China Sea

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1 Introduction

The northern South China Sea (SCS) is a semi-closed marginal sea of the Pacific Ocean with a tropical oceanic climate. The Dongsha waters are located on the northern continental slope of the SCS, and the Dongsha Islands are scattered at a latitude of 20°33′–21°10′N and a longitude of 115°54′–116°57′E, about 260 km from Shantou City and 315 km from the Zhujiang (Pearl River) Estuary. The Dongsha Islands are made up of Pratas lsland, South Vereker Bank and North Vereker Bank, comprising the smallest and closest reefs to the mainland in the north of the SCS, with a total maritime area of 5 000 km^2.

Chinese researchers conducted a series of ecological investigations in the northern SCS from the 1970s (Fan, 1985; Huang et al., 1994; Cai et al., 2002; Ning et al., 2003; Hao et al., 2007; Le et al., 2008). At present, most researchers examining size structure of phytoplankton biomass and productivity concentrate on the coastal areas of the South China Sea, and few examine the waters adjacent to the Dongsha Islands. The results of this study will enrich the data on size-fractionation of phytoplankton of the waters near the Dongsha Islands and explain the causes of the phenomenon.

The phytoplankton community responds quickly to changes in the marine environment. As research continues into marine gas hydrate exploration, the response and corresponding mechanisms of the pelagic ecosystem experiencing seabed methane seepage urgently needs to be recognized and revealed scientifically. A number of anomalous regions of high methane content have been identified in the northern SCS, and scholars have researched the characteristics of the benthic environment, although few studies have focused on the phytoplankton community in the upper waters. Chinese researchers still lack sufficient, original first-hand data about phytoplankton community

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in the natural gas hydrate zone.

The seabed is an ideal place to study the relationship between photosynthetic phytoplankton community structure and a methane-rich environment. This paper has attempted to identify the particularities of photosynthetic phytoplankton community structure characteristics by sampling in the Dongsha natural gas hydrate zone, to find evidence of photosynthetic size-fractionated phytoplankton influenced by marine environmental factors.

This work is based on original, first-hand data that has established an ecological baseline of phytoplankton size structure. It will have important scientific significance for future ecological environmental impact assessments of gas hydrate exploitation in the Dongsha waters.

2 Materials and methods

2.1 *Study date and station*

Field investigations of the size-fractionated phytoplankton biomass and primary production were performed in the Dongsha natural gas hydrate zone of northern SCS from 4 to 9 May, 2013 onboard the R/V *Haiyang IV*, and 16 stations were set up (Fig. 1).

2.2 *Sampling and analytical methods*

Water samples were collected from eight depth layers (0, 30, 50, 75, 100, 150, 200, 300 m) using a SeaBird Electronics (SBE917 Plus) CTD system. Phytoplankton was defined three types: micro (>20 μm), nano (2–20 μm), pico (0.2–2 μm). The implementation method of size-fractionated Chl *a* and primary production is conducted by filtering seawaters through 20 μm, 2 μm, 0.2 μm millipore filters at the first step, respectively.

Chl *a* filters were kept frozen in the dark for 24 h before they were extracted in 90% acetone. The storage process according to the methodology of GB/T 12763.6-2007 (General Administration of Quality Supervision and Inspection and Quarantine of China, 2008b). The Chl *a* content was then measured by the fluorometric method according to Yentsch and Menzel (1963) using a Turner Designs 10-Au-005-CE Fluorometer.

Physical parameters (temperature and salinity) were recorded using a SeaBird Electronics (SBE917 Plus) CTD system. Subsamples were collected from the same Chl *a* layer and stored immediately at –20°C for subsequent analyses of their nutrient contents. The nutrient determination was carried out using a spectrophotometer according to the methodology of GB/T 12763.4-2007 (General Administration of Quality Supervision and Inspec-

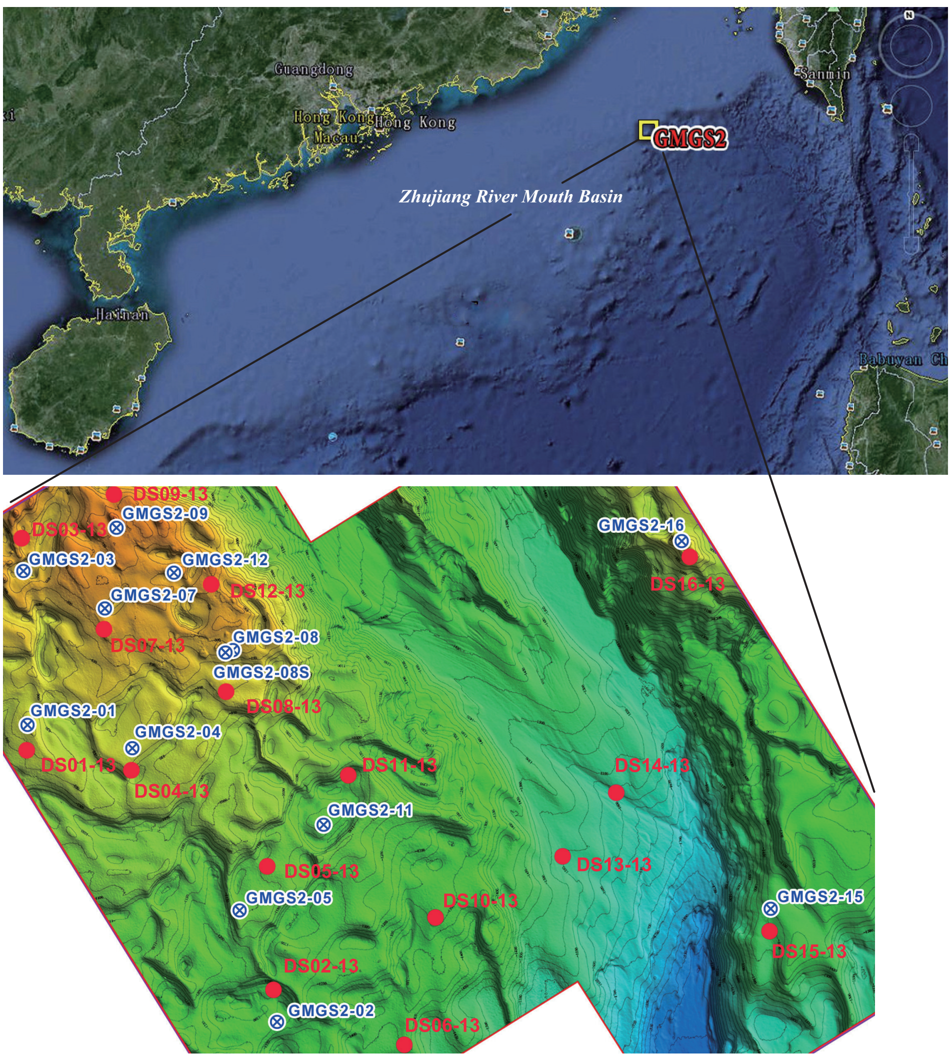

Fig. 1. Map showing locations of GMGS2 drilling sites (called GMGS2-No.) and water sampling stations (called DS No.-13) of biomass and primary production of phytoplankton in the Dongsha waters of northern SCS in May 2013 (Zhang et al., 2014).

tion and Quarantine of China, 2008a).

Primary production was measured by the ^{14}C tracer method (Parsons et al., 1984). Water samples were taken from six different depths. Sampling at stations with euphotic zone were at the surface and the 50%, 25%, 10%, 5%, 1%, and 0.5% daylight level. Acid-cleaned 175 mL polycarbonate bottles (two light and one dark for each depth) were filled with seawater prescreeened through 200 μm mesh. Samples were incubated in situ for 3–6 h after adding 185 kBq of $NaHCO_3$ tracer, and then filtered onto 20 μm, 2 μm, 0.2 μm polycarbonate filters (millipore) respectively at 0.04 MPa vacuum pressure immediately. Filters were soaked in 1 mL 0.1 μmol/mL HCl and allowed to stand in uncapped polycarbonate 20 mL vials 15 min and stored in dark. Ten microliter cocktail (Perkin Elmer) was added after samples taken to labratroy. Total radioactivity counting on filters was performed on a packard Tri-Card 3110TR Liquid Scintillation Counter.

3 Results

3.1 *Characteristics of size-fractionated biomass*

The size-fractionated biomass and percentages of the fractional biomass to the total biomass are shown in Fig. 2 and Fig. 3, respectively. The results showed that picophytoplankton dominated the biomass (82.06%) in the study area, followed by nanophytoplankton (11.68%) and microphytoplankton (6.26%).

The result of vertical biomass distribution indicated that there were significant differences among microphytoplankton, nanophytoplankton and picophytoplankton within the Zeu in the study area. For example, the microphytoplankton was equally distributed vertically, accounting for 2.5%–11.6% of the total biomass proportions, and there was only a small range of change. Nanophytoplankton was mainly observed in the subsurface and middle layer of the Zeu, with a biomass range from 5.0% to 20.1%. Picophytoplankton was mainly distributed in the middle and bottom of the Zeu, with a biomass range from 72.4% to 90.0%, and was mostly above 80%. The vertical trend of percentages of the fractional biomass to the total biomass of picophytoplankton increased from the surface to the bottom of the Zeu, in contrast to the vertical trend of nanophytoplankton (Fig. 3).

3.2 *Characteristics of size-fractionated primary production*

In this study, in view of the fact that the Dongsha waters area is very small and our stations were densely packed and the survey time was short, especially there were exist similar environmental condition of phytoplankton photosynthesis. Three stations (DS04-13, DS02-13 and DS16-13) were selected in our study as representatives of size-fractionated primary production by the ^{14}C tracer method.

The size-fractionated primary production and the percentages of fractional primary production to total primary production are shown in Figs 4 and 5, respectively. The results showed that in the study area picophytoplankton dominated total primary production (81.48%), followed by nanophytoplankton (13.22%) and microphytoplankton (5.30%).

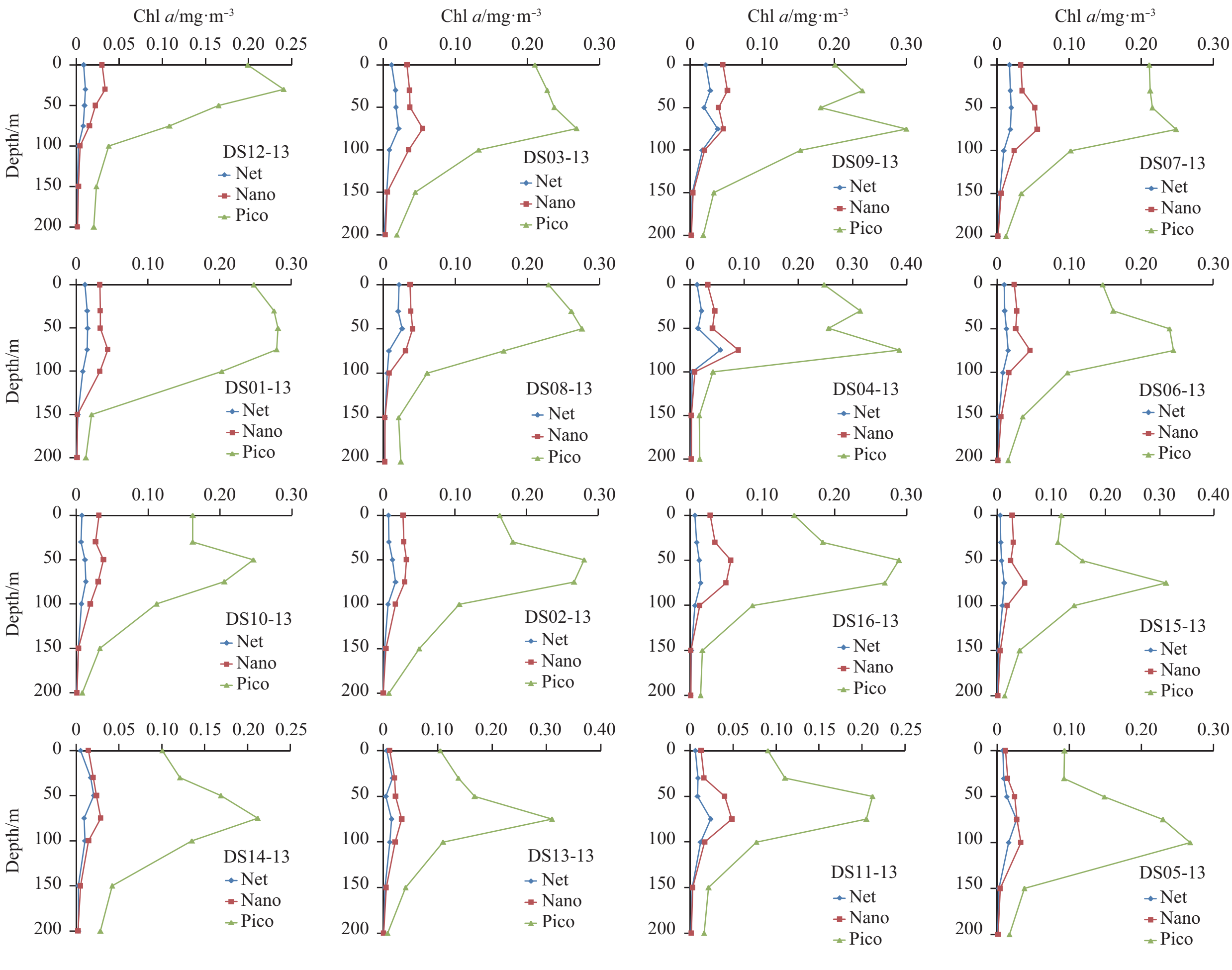

Fig. 2. Vertical distribution of phytoplankton size-fractionated biomass in the Dongsha waters.

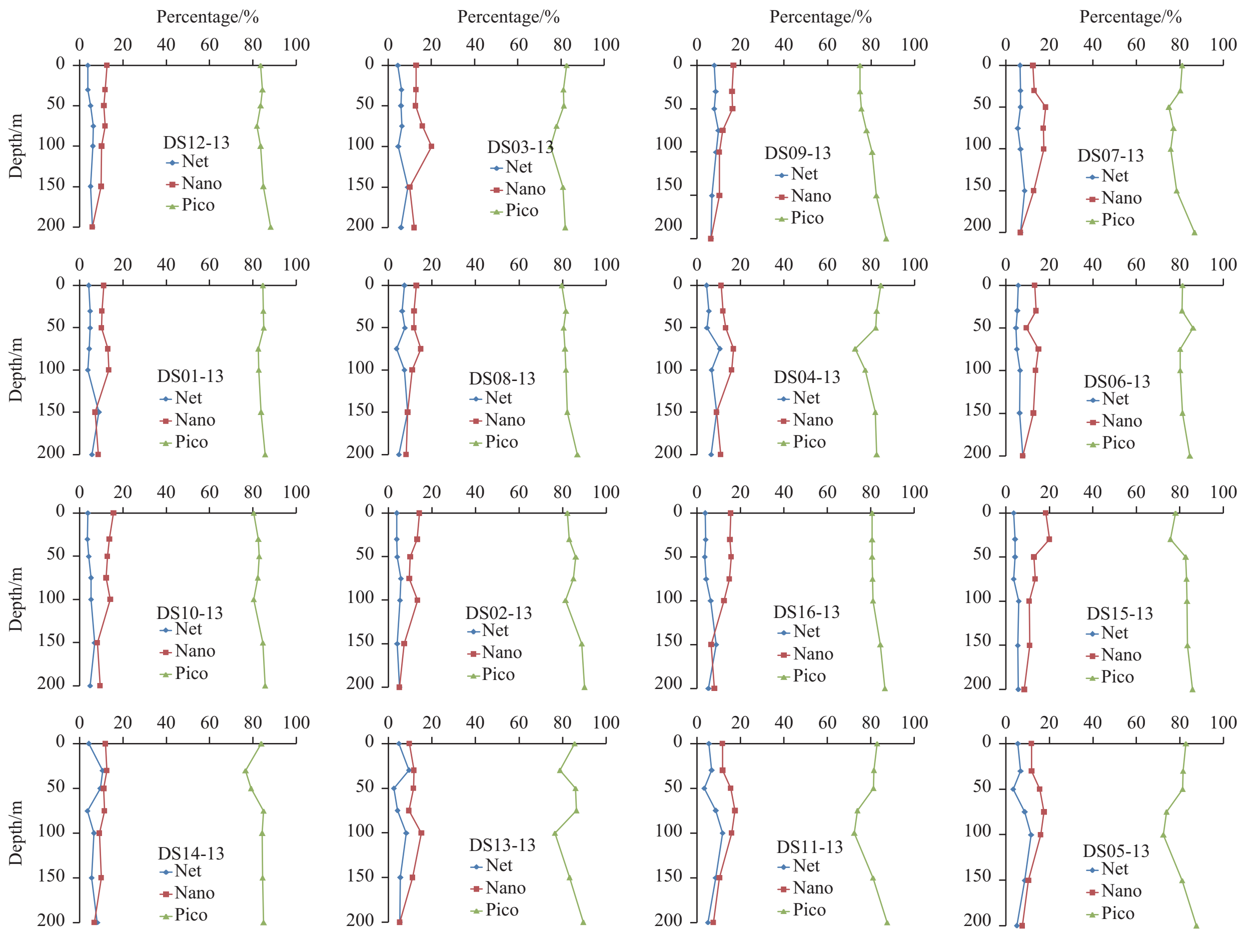

Fig. 3. The percentage of phytoplankton size-fractionated biomass in total biomass in the Dongsha waters.

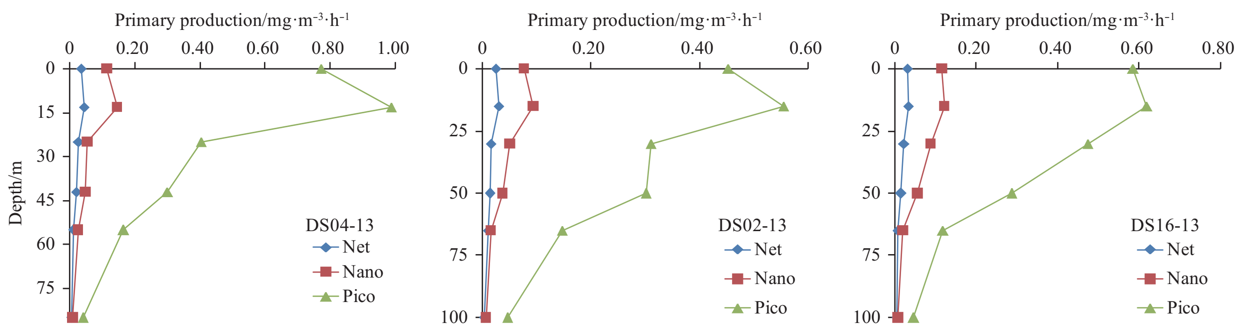

Fig. 4. Vertical distribution of size-fractionated primary production in the Dongsha waters.

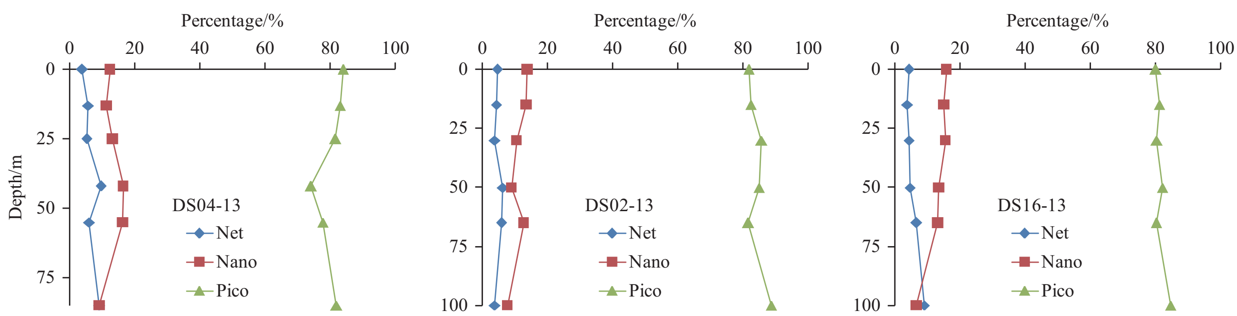

Fig. 5. The percentage of phytoplankton size-fractionated primary production in total production in the Dongsha waters.

The results of vertical primary production distribution indicated that there were significant differences among microphytoplankton, nanophytoplankton and picophytoplankton within the Zeu in our study area. For example, microphytoplankton was vertically distributed equally, accounting for primary production proportion in 3.8%–9.5%, changed in a small range. Nanophytoplankton was mainly observed in the subsurface and middle layer of the euphotic zone with a primary production range of 7.7%–16.5%. Picophytoplankton was mainly distributed in the middle and bottom of the Zeu with a primary production range of 74.0%–88.5%, and was generally higher than 80%.

The trend of vertical distribution of size-fractionated primary production was similar to that of size-fractionated biomass.

4 Discussion

4.1 *Relationship with enviromental factors*

Spearman correlation analysis between size-fractionated biomass, primary production and environmental factors was carried out in May 2013 (Table 1). The results showed there was a positive correlation between Chl *a* and temperature, pH, but a negative correlation between Chl *a* and silicate, phosphate for microphytoplankton, nanophytoplankton and picophytoplankton. In addition, as few stations measured methane, phytoplankton biomass and methane non-performance a significant correlation.

There was a positive correlation between primary production and temperature but a negative correlation between primary production and salinity, phosphate for microphytoplankton, nanophytoplankton and picophytoplankton.

4.2 *Explain of results*

The Chl *a* biological baseline information in the Dongsha natural gas hydrate zone is shown in Table 2 and Fig. 6.

The evaluation criteria for Chl *a* biological baseline control chart (Fig. 6) are:

(1) If the drop in sample value is between the upper and lower warning limits, it indicates that the quality of all the samples analysed is reliable and the results are at normal levels under the environmental conditions and are not disturbed by abnormal environmental factors.

(2) If the placement of the sample value is above the upper and lower warning limits, but still between the upper and lower control limits, it indicates that the data quality of the sample analysis has deteriorated and the measurement results tend to be out of control under the environmental conditions.It is necessary to perform a preliminary cause analysis to check whether the sample has been disturbed by abnormal environmental factors.

(3) If the drop in sample value is off the upper and lower control lines, it indicates that the measurement results have deteriorated and have been completely out of control under the environmental conditions. The cause should be identified immediately to determine whether the sample was polluted by methane during the mining period.

Table 1. Correlations between biomass and primary production of phytoplankton and environmental factors in the Dongsha waters

Parameters	Statistic	T	S	SiO_3-Si	PO_4-P	NH_3-N	CH_4	pH
Chl *a*-Net	Correlation	0.577**	−0.118	−0.620**	−0.598**	0.202	0.138	0.593**
	Sig.(2-tailed)	0.000	0.228	0.000	0.000	0.087	0.669	0.000
	n	107	107	53	102	73	12	107
Chl *a*-Nano	Correlation	0.658**	−0.023	−0.699**	−0.700**	0.143	0.123	0.664**
	Sig.(2-tailed)	0.000	0.818	0.000	0.000	0.229	0.704	0.000
	n	107	107	53	102	73	8	107
Chl *a*-Pico	Correlation	0.693**	−0.060	−0.726**	−0.769**	0.145	0.117	0.710**
	Sig.(2-tailed)	0.000	0.540	0.000	0.000	0.220	0.717	0.000
	n	107	107	53	102	73	12	107
PP-Net	Correlation	0.746**	−0.760**	−0.608	−0.635**	−0.227	–	0.401
	Sig.(2-tailed)	0.001	0.001	0.200	0.008	0.399	–	0.123
	n	16	16	6	16	16	–	16
PP-Nano	Correlation	0.691**	−0.691**	−0.679	−0.569*	−0.440	–	0.413
	Sig.(2-tailed)	0.003	0.003	0.138	0.021	0.088	–	0.112
	n	16	16	6	16	16	–	16
PP-Pico	Correlation	0.686**	−0.691**	−0.579	−0.581*	−0.348	–	0.373
	Sig.(2-tailed)	0.003	0.003	0.228	0.018	0.187	–	0.154
	n	16	16	6	16	16	–	16

Note: ** Correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed). The initial Chl *a*-Net stand for Chl *a* of netphytoplankton, Chl *a*-Nano Chl *a* of nanophytoplankton, Chl *a*-Pico Chl *a* of Picophytoplankton, PP-Net primary production of netphytoplankton, PP-Nano primary production of nanophytoplankton, PP-Pico PP of Picophytoplankton, and *n* number of samples.

Table 2. Baseline data of size-fractionated biomass (mg/m^2) in the Dongsha natural gas hydrate zone

Type	Water column Chl *a* (*n*=16)	Microphytoplankton Chl *a* (*n*=16)	Nanophytoplankton Chl *a* (*n*=16)	Picophytoplankton Chl *a* (*n*=16)
Baseline	30.64	1.82	3.96	24.83
Upper warning line	40.37	2.93	5.70	32.54
Upper control line	45.24	3.49	6.58	36.39
Lower warning line	20.90	0.70	2.22	17.12
Lower control line	16.04	0.15	1.35	13.27

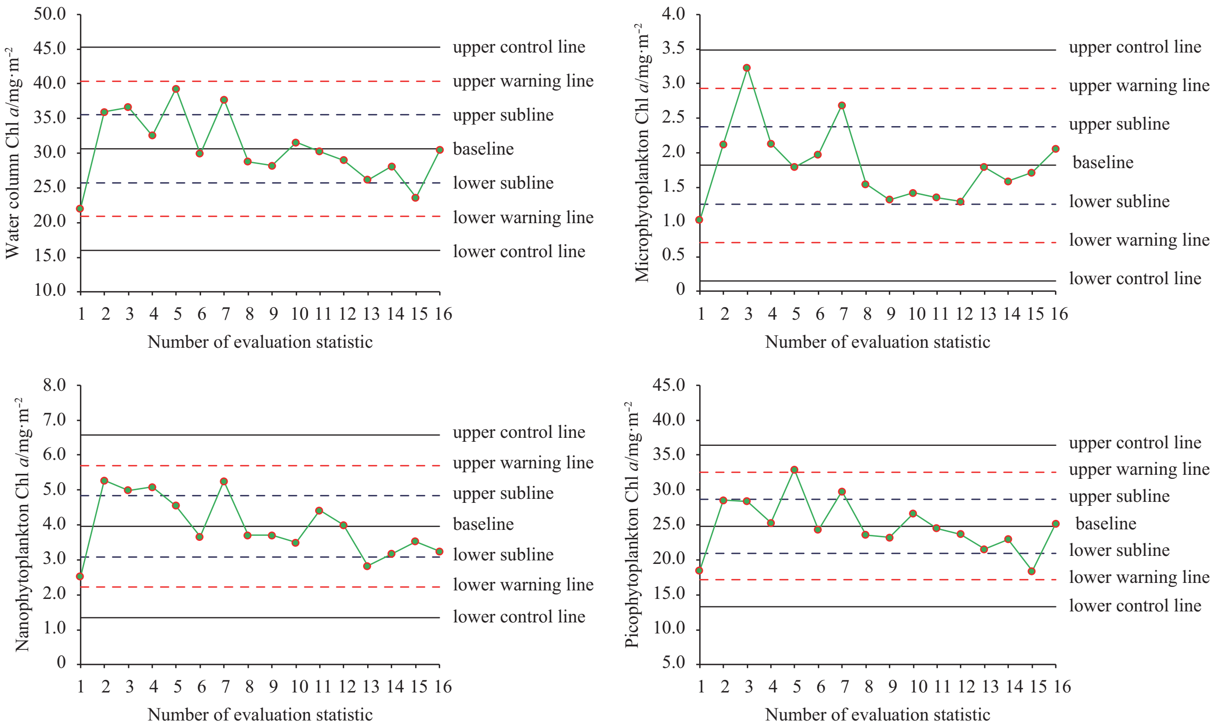

Fig. 6. Baseline graph of size-fractionated biomass (mg/m^2) in the Dongsha natural gas hydrate zone.

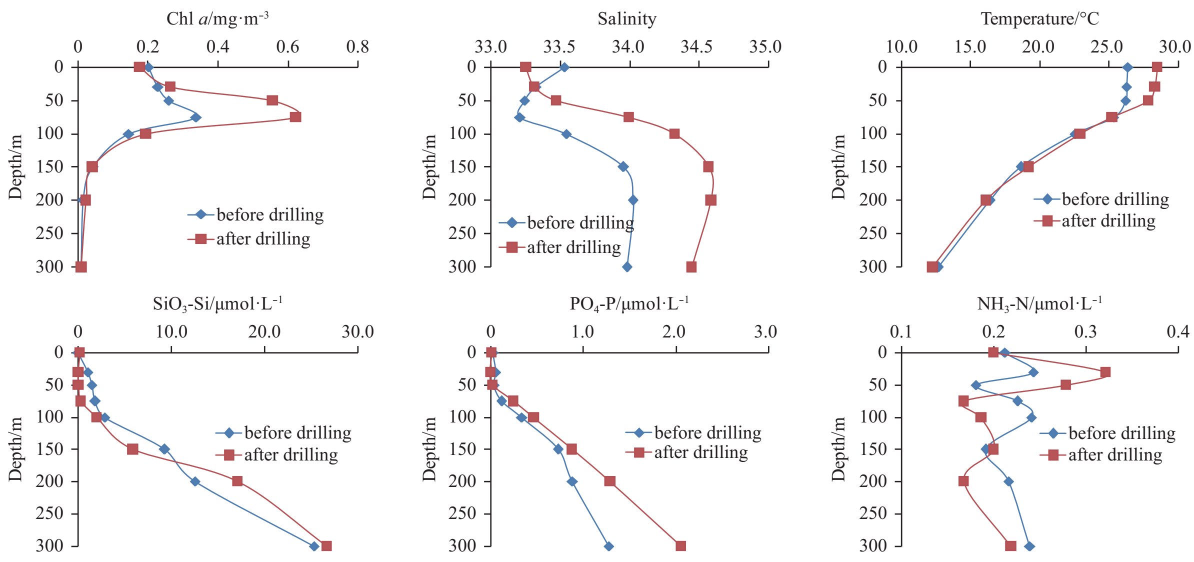

Fig. 7. Comparison of environmental parameters before (May) and after (October) drilling in 2013.

Figure 7 shows changes in physical, chemical and biological parameters before and after drilling in 2013. For example the maximum Chl *a* value was 0.62 mg/m^3 after drilling, 1.9 times the level before drilling. Chl *a* in the water column after drilling was 48.25 mg/m^2; before drilling its level in the water column was 31.67 mg/m^2; and after drilling it was 1.5 times greater.

On-site monitoring and shipborne observation systems have been run by scientists in the Gulf of Mexico, who carried out studies of ten years of marine satellite data. They found that offshore hydrocarbon seeps—and perhaps other types of deep ocean vents and seeps at depths exceeding 1 000 m—may transport nutrients and hydrocarbons to the subsurface. These may result from plume-generated upwelling, and influence biogeochemistry and productivity of the overlying water column, resulting in a significant increase in the concentration of Chl *a* in upper waters (D'souza et al., 2016). D'souza found that the total Chl *a* (28.2 mg/m^2) in the water column in the Gulf of Mexico was about 1.3 times that of the background area (22.3 mg/m^2). The average content of the maximum layer (0.66 mg/m^3) was more than twice that of the background area (0.29 mg/m^3). The Deep Chlorophyll Maximum (DCM) in the seep area (82 m) was more than 18 m that of the background area (100 m) (Fig. 8). In addition, Chl *a* at seep stations was skewed towards the higher end of distribution, with higher kurtosis values suggesting that the increase in variance strongly deviated from the biological baseline and exceeded the control limit, rather than showing multiple,

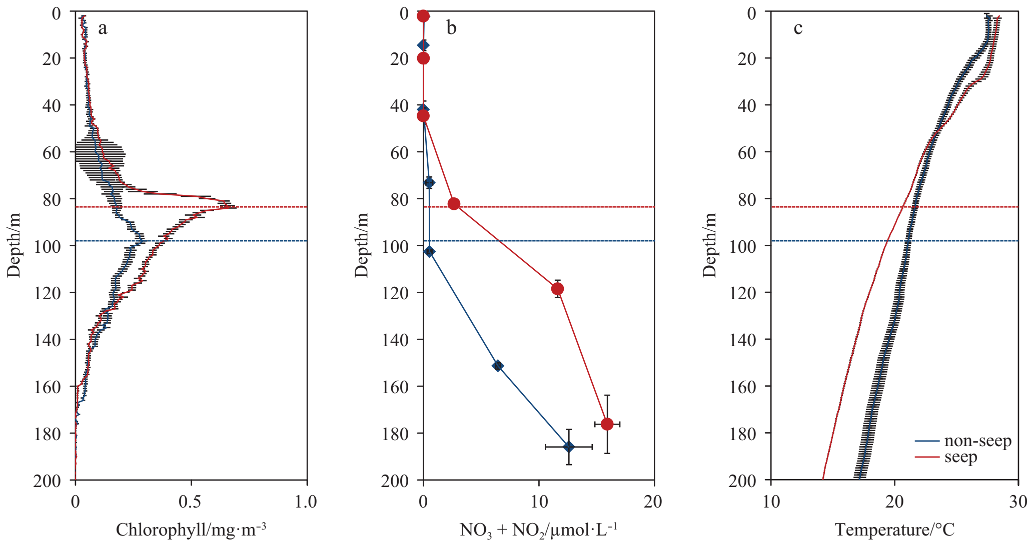

Fig. 8. Water column profiles of Chl *a*, nutrients and temperature above seep and background sites. a-c. Average seep (red) and non-seep background (blue) (D'souza et al., 2016).

modest increases.

The current study has been able to document clearly that the leakage of oil and gas can affect the photosynthetic phytoplankton biomass of the upper water. The turbulence from the natural gas leaking zone can raise the bottom water into the rising bubbles and bring cold water containing rich nutrients to the thermocline from deep seeps (Sauter et al., 2006). This plume-generated upwelling above seeps can generate a bottom-up effect on the photosynthetic organ of the entire water column below the surface, similar to the influence of eddy currents driving upflows in the subtropical waters, bringing rich nutrients that affect phytoplankton biomass and productivity (McGillicuddy et al., 2007).

The environmental parameters after drilling in Dongsha waters in 2013 appear to coincide with the findings in the Gulf of Mexico (Figs 7 and 8), but there is insufficient evidence to show that results caused by methane release. This is because whether methane releases or seasonal changes, or other marine environmental factors, need to be verified with much more informative data, and we also need more data to improve the biological baseline.

The Dongsha gas hydrate zone is a complex and sensitive ecological environment, affected by the stratified waters and the differing vertical distributions of light, temperature, salinity and nutrients. These factors contribute to the different distributions of size-fractionated phytoplankton. The results show that picophytoplankton was the main contributor to phytoplankton primary productivity; nanophytoplankton was a minor contributor; and microphytoplankton the smallest contributor.

Picophytoplankton typically contains *Prochlorococcus*, *Synechococcus* and picoeukaryotes. It is one of the most important primary producers in oligotrophic waters and its contribution to the biomass may be more than 80% (Fig. 3). Many picophytoplankton are small, and they have a fast and highly sensitive response to marine environmental changes through adjustments in cell size, abundance and pigment content (Zhang et al., 2008).

The most critical ecological factor for picophytoplankton growth is temperature (Huang et al., 2003): a low value can be fatal. In general, picophytoplankton abundance increases as temperature rises (Table 1), but its cell division is strictly temperature controlled. Moore et al. (1995) found the best temperature for *Prochlorococcus* growth to be 24°C; if the temperature was higher than 28°C and below 12.5°C, *Prochlorococcus* was incapable of growing. Although there was a positive correlation between Chl *a* and temperature, the temperature has little effect on the distribution of phytoplankton in the area investigated in such short time and small scale. The effect of temperature on the vertical distribution of phytoplankton is mainly reflected on the water layer and stability of the thermocline. The positive correlation between temperature and biomass of phytoplankton is mainly because the reduction of phytoplankton biomass in the water with low temperature is due to the reduction of light intensity, instead of being directly impacted by temperature.

Nutrients have an overall impact on phytoplankton. Picophytoplankton has been seen to be able to develop in large numbers in a sea with extremely poor nutrition. The main reason is that very small cell volume and large surface area enable it to compete for nutrients (Campbell and Vaulot, 1993). The smaller the cell size, the lower the half-saturation constant. Picophytoplankton, with its smaller cell size, has an advantage over other phytoplankton when competing in low-nutrient environments, especially oligotrophic open oceans. *Prochlorococcus*, for example, one of the smaller picophytoplankton that exists the upper limit of nutrients, often appears in large numbers in oligotrophic open oceans. Results of the Arabian Sea and East China Sea survey showed that *Prochlorococcus* was only found in waters in which TIN<3 μmol/L and PO_4-P<0.4 μmol/L (Campbell et al., 1998; Jiao, 2006).

A study by Fang et al. (2006) into phytoplankton growth and its response to phosphorus absorption changes was conducted under culture conditions of light and nutrients. The results indicated that phosphate uptake rates clearly increased at a high phosphate level under high irradiance (100% natural irradiance), showing phytoplankton growth was strongly phosphate limited. The cell densities of nanophytoplankton and *Synechococcus* sp. also clearly increased. Picophytoplankton seems to be adapted to a low phosphate level. *Prochlorococcus* has a low demand for phosphorus, and can grow well in areas where phosphate is deficient, such as the Mediterranean Sea and the Sargasso Sea (Cotner et al., 1997; Thingstad and Rassoulzadegan, 1995).

It has been reported that the half-saturation constant of NO_3-

N uptake by marine microphytoplankton is 0.1–6.5 μmol/L and that of PO_4-P is 0.12–0.55 μmol/L (Chen and Qian, 1992). In this study, the average concentration of PO_4-P within 100 m was 0.11 μmol/L (n=16), significantly lower than the half-saturation constant of microphytoplankton. Such a low concentration of PO_4-P is likely to limit the growth of nanophytoplankton. In our study, the contribution of microphytoplankton and nanophytoplankton to biomass was only 6.26% and 11.68%, respectively. Our result indirectly confirmed this conjecture.

It can be seen that PO_4-P is one of the most important factors affecting different phytoplankton species in Dongsha waters, and different phytoplankton species have different adaptation to phosphate level. This conclusion is consistent with the findings of Hong et al. (1997) and Huang et al. (1997) in the Taiwan Strait.

With respect to primary production, it is generally believed that if a certain area is dominated by large nanophytoplankton and microphytoplankton cells, the corresponding primary productivity of this area is also higher, and vice versa.

In terms of size structure, the results of the Taiwan Strait study showed that microphytoplankton and nanophytoplankton gradually decreased from north to south, and that picophytoplankton gradually increased from north to south, resulting in a gradual decline of the primary productivity from north to south (Kang, 2009). In our study, primary productivity was at a low level in the northern SCS compared with the Taiwan Strait, in line with that study.

The result of vertical biomass distribution indicated that there were many differences among microphytoplankton, nanophytoplankton and picophytoplankton within the Zeu. For example, microphytoplankton was mainly distributed evenly from up to down; nanophytoplankton appeared in the subsurface and in the middle of the euphotic layer; and picophytoplankton stayed in the middle and at the bottom of the Zeu (Fig. 9). Factors affecting the vertical distribution of phytoplankton were very complex: irradiation and the stability of waters are important in regulating the vertical distribution of size-fractionated phytoplankton.

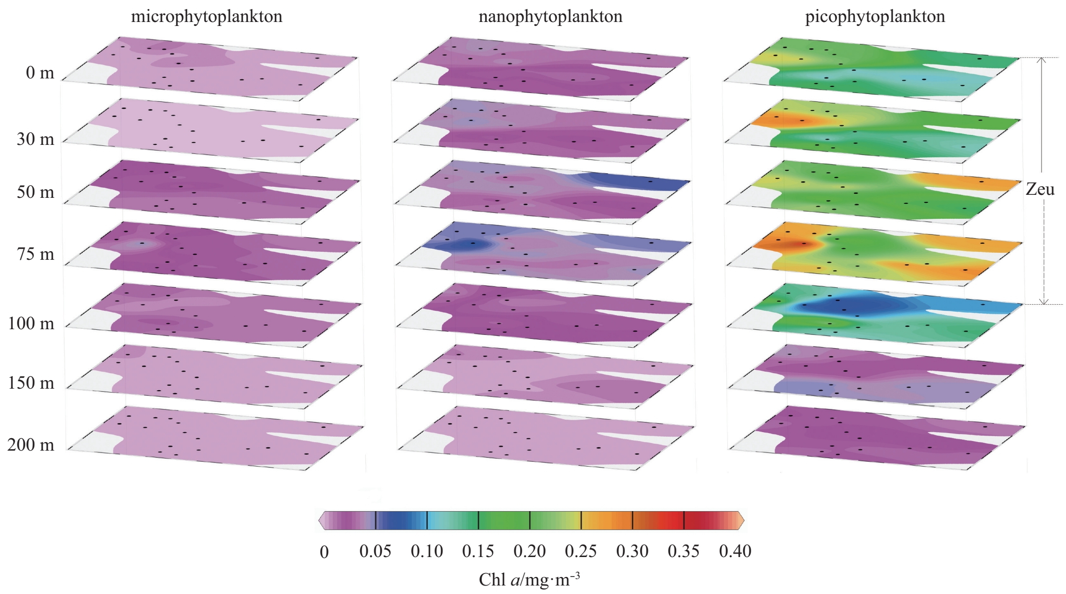

Fig. 9. Horizontal distribution of size-fractionated biomass.

As shown in Fig. 10, the total Chl *a* of phytoplankton is in the range of 0.00–0.53 mg/m³ with a relatively small change, and DCM located in 30–100 m with a large amount of change. From 100 m to 200 m, Chl *a* gradually decreased and was uniformly distributed in the same water layer.

It can be seen from Fig. 11 that the high values of photosynthetic rate of Chl *a* and primary productivity appear in the subsurface in Dongsha waters. This result is similar to the findings of Huang et al. (1997) in the Taiwan Strait and Ning and Worrall (1991) in the East China Sea.

The analysis showed that the phenomena of SCMLs and SPMLs depend first on the thermocline, euphotic depth and water stability, followed by the adaptability distribution of phytoplankton to light and the distribution gradient of nutrients in the thermocline to the growth effect of different phytoplankton. The thermocline will prevent the transport of eutrophic water from the bottom to the surface, with the result that the eutrophic water only diffuses upwards through the lower boundary of the thermocline. A gradient of nutrient distribution with increasing depth is thus formed below the thermocline (that is, the muta-

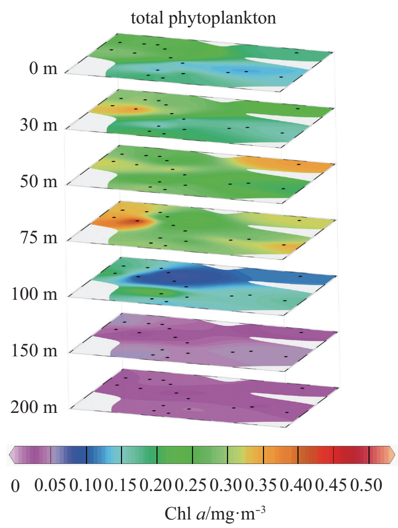

Fig. 10. Horizontal distribution of water column Chl *a*.

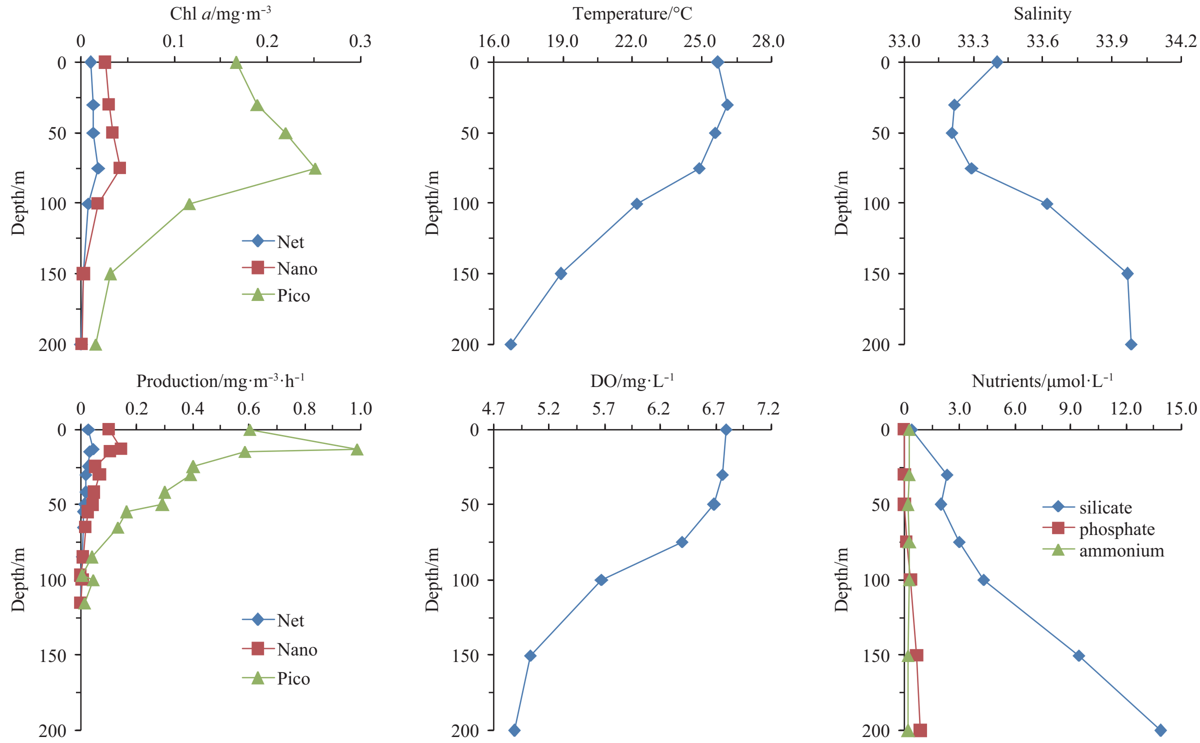

Fig. 11. Vertical distribution of physical-biochemical indexes coupled all stations (n=16) in the Dongsha waters.

tion zone that provides sufficient nutrients for the growth of phytoplankton) (Shen et al., 2010; Cullen, 2015).

Although light is plentiful above the thermocline, vertical convection of seawater is not ideal. Nutrient consumption by phytoplankton accelerates the oligotrophic effect, which in turn inhibits the growth of microphytoplankton and nanophytoplankton. Although the nutrient level increases slightly below the thermocline, light conditions and photosynthesis rate are forced to decrease rapidly, resulting in survival failure of microphytoplankton and nanophytoplankton (Figs 11–13).

In the Zeu, the range of DCM is located at depths of 10%–1% daylight level (Fig. 2), which indicates that some phytoplankton are adapted to a low light environment. Conditions of the mixed layer being deeper than the Zeu will be very detrimental to picophytoplankton photosynthesis in the Zeu. The dark adaptation of phytoplankton to light requires a certain time, and the time of stay and adaptation will affect its growth in the Zeu, resulting in a decrease in phytoplankton abundance. In this study, the Zeu depth (100 m) was deeper than the mixed layer (60 m). Therefore, weak light reaching the picophytoplankton was not a significant restriction.

The literature (Jiao, 2006) shows that there is a significant correlation between the nutrient thermocline layer and the micromaximum abundance layer, where the picophytoplankton have an absolute advantage.

In the typical oligotrophic waters of the SCS, one of the prominent features of the vertical distribution of *Prochlorococcus* is that the abundance maxima lie below the subsurface (usually

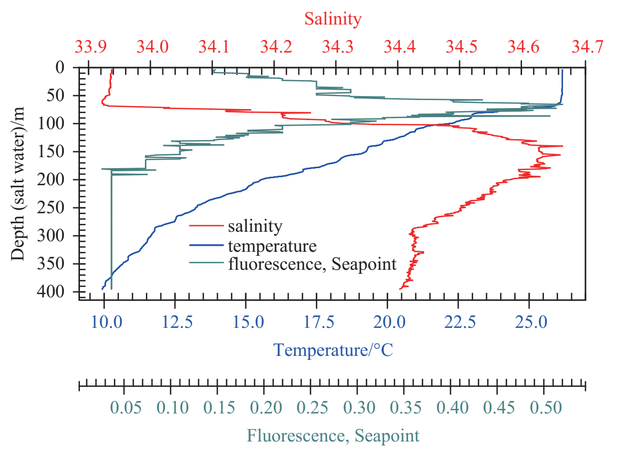

Fig. 12. Profile of temperature (°C) and salinity and fluorescence (mg/m³) in DS02-13.

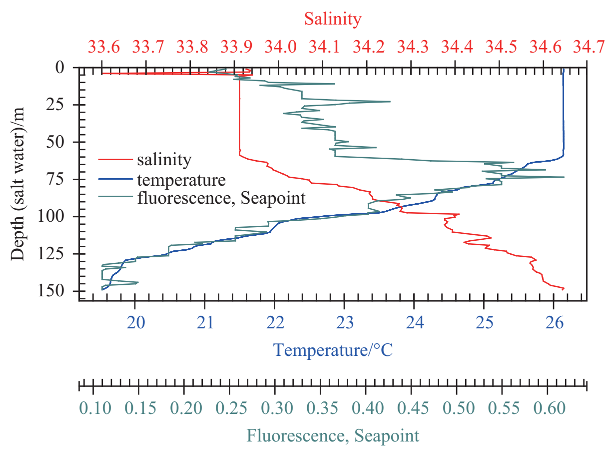

Fig. 13. Profile of temperature (°C) and salinity and fluorescence (mg/m³) in DS04-13.

between 50 m and 75 m), which is consistent with the conclusion of Ning et al. (2003) in 1999 in the northern SCS.

DuRand et al. (2001) reported that nanophytoplankton were mainly distributed in the subsurface or the middle of the Zeu, while the maximum layer of picophytoplankton (including *Prochlorococcus*) was usually found at the bottom of the Zeu.

The photosynthetic rates between picophytoplankton and nanophytoplankton were compared by Glover et al. (1985), who concluded that living on the surface layer is not suited to picophytoplankton, which is more suited to living in places where the light intensity is weak, such as the bottom of the Zeu. Their study proposed the hypothesis that picophytoplankton is a "negative plant". Picophytoplankton growth is affected by photoinhibition in the surface, while it has a higher utilization rate of the blue-green light with weak energy in the euphotic layer. The contribution of picophytoplankton to photosynthesis increases with depth in the euphotic layer (Stockner and Antia, 1986). The results of the present study further confirmed the hypothesis that picophytoplankton is a "negative plant".

5 Summary

We have comprehensively analysed the distribution characteristics and environmental factors influencing biomass and primary productivity size structure in Dongsha waters in the northern SCS. This analysis was based on the biological data from Dongsha waters, coupled with marine physical-biochemical indexes. The main findings are:

(1) Low nutrients, low Chl *a* and primary productivity characteristics were observed in the Dongsha waters. The phenomena of SCMLs and SPMLs were significant in the study area.

(2) The phenomena of SCMLs and SPMLs first depended on the thermocline and euphotic depth and water stability, followed by the adaptability distribution of phytoplankton to light and the distribution gradient of nutrients in the thermocline to different phytoplankton species growth effects.

(3) There were significant differences in the size-fractionated biomass and primary production, indicating the order picophytoplankton>nanophytoplankton>microphytoplankton from the perspective of biomass and degree of contribution to productivity.

(4) Three different size-fractionated phytoplankton species assemblages showed different vertical distribution of biomass. Microphytoplankton was distributed evenly within the Zeu; nanophytoplankton was mainly distributed in the subsurface or in the middle of the Zeu; and picophytoplankton was mainly distributed in the middle or bottom of the Zeu.

(5) Correlation analysis showed that biomass had a significant positive correlation with temperature and pH, and was negatively correlated with silicate and phosphate. Primary production of phytoplankton was positively correlated with temperature, and negatively correlated with salinity and phosphate. The phytoplankton biomass did not show a significant correlation with methane owing to our study took fewer samples from sites where methane was present.

(6) Picophytoplankton belongs to the"negative plant" category, having a dominant advantage in low-latitude, tropical oligotrophic waters. The contribution of phytoplankton to biomass and primary productivity in spring was also controlled by nutrient, especially PO_4-P, which was one of the most important factors influencing the difference in size-fractionated phytoplankton in the Dongsha waters. In addition, illumination and the euphotic layer played important roles in controlling the vertical distribution of marine size-fractionated phytoplankton.

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