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Comparison of photosynthetic pigments and phytoplankton assemblages in two types of coastal regions in Southeast Asia-Indonesian Throughflow and river estuary

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Abstract

Water samples were collected in order to study the spatial variation of photosynthetic pigments and phytoplankton community composition in the Lembeh Strait (Indonesia) and the Kelantan River Estuary (Malaysia) during July and August 2016, respectively. Phytoplankton photosynthetic pigments were detected using high performance liquid chromatography combining with the CHEMTAX software to confirm the Chl *a* biomass and community composition. The Chl *a* concentration was low at surface in the Lembeh Strait, which it was 0.580–0.682 μg/L, with the average (0.620±0.039) μg/L. Nevertheless, the Chl *a* concentration fluctuated violently at surface in the Kelantan River Estuary, in which the biomass was 0.299–3.988 μg/L, with the average (0.922±0.992) μg/L. The biomass at bottom water was higher than at surface in the Kelantan River Estuary, in which the Chl *a* concentration was 0.704–2.352 μg/L, with the average (1.493±0.571) μg/L. Chl *b*, zeaxanthin and fucoxanthin were three most abundant pigments in the Lembeh Strait. As a consequence, phytoplankton community composition was different in the two study areas. In the Lembeh Strait, prasinophytes (26.48%±0.83%) and *Synechococcus* (25.73%±4.13%) occupied ~50% of the Chl *a* biomass, followed by diatoms (20.49%±2.34%) and haptophytes T8 (15.13%±2.42%). At surface water in the Kelantan River Estuary, diatoms (58.53%±18.44%) dominated more than half of the phytoplankton biomass, followed by *Synechococcus* (27.27%±14.84%) and prasinophytes (7.00%±4.39%). It showed the similar status at the bottom water in the Kelantan River Estuary, where diatoms, *Synechococcus* and prasinophytes contributed 64.89%±15.29%, 16.23%±9.98% and 8.91%±2.62%, respectively. The different phytoplankton community composition between the two regions implied that the bottom up control affected the phytoplankton biomass in the Lembeh Strait where the oligotrophic water derived from the West Pacific Ocean. The terrigenous nutrients supplied the diatoms growing, and pico-phytoplankton was grazed through top down control in the Kelantan River Estuary.

Key words: phytoplankton, pigment, Lembeh Strait, Kelantan River Estuary

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1 Introduction

The phytoplankton is the major primary producer which contributes about half of the global primary production through no more than 1% of the autotrophic standing stock (Falkowski, 2012; Field et al., 1998). The standing biomass and dynamic variation of phytoplankton was the essential question in both marine biology and biological oceanography studies. In addition, the composition of phytoplankton in natural condition was great variable no matter at the population or community level (Fasham, 2003).

As far as the phytoplankton was concerned, the community composition and succession responded to the nutrients, light and other environmental factors. The carbon fixation capacity varied among different phytoplankton groups, e.g., the diatoms were the major contributor to the new production (Eppley and Peterson, 1979; Goldman, 1993), and the pico-phytoplankton distributed mainly in the oligotrophic water (Chisholm et al., 1988; Fogg, 1986). On the other hand, the ecological roles were also different in the food web or the microbial loop for different phytoplankton groups (Azam et al., 1983), and their efficiency in the biological pump was different deservedly (Eppley and Peterson, 1979; Michaels and Silver, 1988). In the tropical South China Sea

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(SCS) basin, where the nutrients are oligotrophic (Chen et al., 2010; Chen, 2005; Chu and Fan, 2001; Wong et al., 2002), the phytoplankton biomass and primary production are usually low. The status is generally affected by both the bottom-up control induced by permanent stratification of the water column (Wong et al., 2007) and the top-down control through the food web (Sherr and Sherr, 1994) or microbial loop (Azam et al., 1983).

As the ligament of West Pacific Ocean (WP) and the eastern Indian Ocean, there must be the most complex current system in the Southeast Asia, especially around the Indonesia Islands. Wyrtki (1961) firstly defined the Indonesian Throughflow (ITF) as the result of the gradients between higher sea level in the West Pacific and lower sea level in the eastern Indian Ocean (Wyrtki, 1961). The currents velocity of ITF could be more than 15×10^6 m^3/s (Gordon, 2005) with the heat flux 0.5–1.0 petawatts from the Pacific into the Indian Ocean (Vranes et al., 2002). The temperature-salinity properties of the ITF are cooler and fresher water mass which could penetrate through the island waterway into the Indian Ocean (Gordon, 2005). The purpose of this study is to make comparison between two typical coastal ecosystems in the Southeast Asia which influenced by the oceanic or riverine water respectively.

The hypothesis was that as the distribution and community composition of phytoplankton in the SCS was spatial variable, these are phytoplankton patches, especially in the coastal regions with or less terrigenous nutrients. The scientific questions had been processing from two portions in the present study, that was (1) the distribution of phytoplankton in the coastal regions in the Southeast Asia; (2) the limit factors for spatial variation of major phytoplankton functional types (PFTs) in the study areas, in brief, whether the oligotrophic Indonesian Throughflow and riverine affected the phytoplankton biomass and community composition in the two different coastal systems.

2 Materials and methods

2.1 *Study area*

Two cruises were carried out in the Kelantan River Estuary, Malaya, Malaysia and in the Lembeh Strait, North Sulawesi, Indonesia during 29–30 July and 20–21 August 2015, respectively (Fig. 1). Among all the stations with hydrological data, there were 11 and 10 pigments samples at the surface or bottom water in the Kelantan River Estuary, and there were four pigments samples in the Lembeh Strait at surface layer. The stations information was showed in Table 1.

2.2 *Sampling*

Sea-Bird SBE–911 Plus V2 conductivity-temperature-depth (CTD) system was deployed to acquire hydrographic parameters. Seawater samples for measurement of phytoplankton pigments by high-performance liquid chromatography (HPLC) were collected by CTD-mounted rosette assemblies with twelve 2.5 L Niskin bottles (General Oceanic Inc.) during the deployment.

2.3 *Pigments*

Seawater samples (0.2–0.5 L) for pigment analysis were filtered onto Whatman GF/F filters of 25 mm diameter under gentle

Table 1. The stations information in this study

Cruise	Name	North latitude/(°)	East longitude/(°)	Temperature/°C	Salinity
Lembeh Strait	A12	1.47	125.23	27.90	33.90
	A13	1.48	125.24	28.00	34.50
	A16	1.50	125.25	28.70	33.90
	A9	1.43	125.19	28.90	33.90
Kelantan River Estuary	C1	6.20	102.27	30.81	31.65
	KW14	6.21	102.23	30.52	4.00
	KW15	6.22	102.24	31.41	8.57
	KW16	6.23	102.24	31.84	21.16
	KW17	6.25	102.24	30.68	21.78
	KW2	6.20	102.29	31.30	24.58
	KW3	6.22	102.30	30.60	32.15
	KW4	6.25	102.30	30.62	27.37
	KW5	6.22	102.29	30.62	32.61
	KW7	6.21	102.26	30.79	32.50
	KW8	6.24	102.27	30.29	32.18

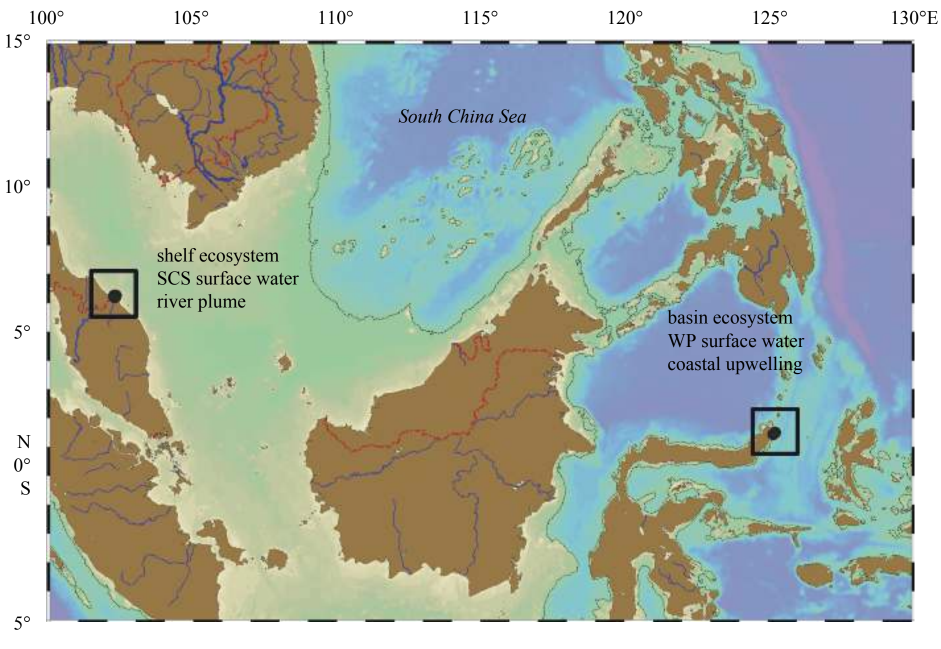

Fig. 1. Map of the study area and the location of sampling stations during cruises.

vacuum (<0.02 MPa). The filters were wrapped with aluminum foil and frozen stored in liquid nitrogen until analysis. When transported to the laboratory the frozen samples were displaced in freezer (–80°C). The pigment concentrations were detected using High Performance Liquid Chromatography (HPLC) following standard method (Zapata et al., 2000). The frozen filter was soaked in 1 mL N, N-dimethylformamide (DMF) extraction in a freezer (–20°C) for 2 h (Furuya et al., 1998). The extractions were then filtered through Whatman GF/F filters of 13 mm diameter (Swinnex® filter holder) to clean the debris and then mixed with ammonium acetate solution (1 mol/L) in equal proportion. A quarter of each mixture was injected into a Shimadzu LC20A-DAD HPLC system fitted with a 3.5 μm Eclipse XDB C_8 column (100 mm×4.6 mm; Agilent Technologies). Quantification was confirmed by the standards manufactured by Danish Hydraulic Institute (DHI) Water and Environment, Hørsholm, Denmark.

2.4 *CHEMTAX*

The chemical taxonomy program, CHEMTAX, was applied under MATLAB (the MathWorks, Inc., Natick, Massachusetts) platform to acquire the relative contributions of nine phytoplankton groups to total Chl *a*. Thirteen pigment markers were introduced to quantify each fraction of the total Chl *a* pool of nine phytoplankton groups, including dinoflagellates (Dino), diatoms (Diat), haptophytes_8 (Hapt_8), haptophytes_6 (Hapt_6), chlorophytes (Chlo), cryptophytes (Cryp), *Prochlorococcus* (Proc), *Synechococcus* (Syne) and prasinophytes (Pras). The ratios of initial inputting pigment to Chl *a* (F_{input}) followed the processes using in previous studies (Table 2) with the addition of Dv-Chl *a* for *Prochlorococcus* for the SCS (Table 3) (Mackey et al., 1996). According to the rule of running CHEMTAX mentioned by Latasa (2007), successive runs were necessary to gain the convergence between input and output ratio (F_{output}). The essential rules were obeyed to the modification and optimizing of the CHEMTAX running in the SCS (Wang et al., 2015a).

2.5 *Statistic analysis*

Figures were drawn by OriginPro 9.0 (OriginLab Corporation, Northampton, MA, USA). The independent-samples *t*-test, One-Way ANOVA and Duncan's multiple range test was dealt by PASW® Statistics 17.0 software.

3 Results

3.1 *Hydrology*

The distribution of monthly climatology surface temperature and salinity in the southern SCS and adjacent area showed the remarkable differences, no matter in horizontal nor in seasonal distribution (Fig. 2). For the sea surface temperature, the seasonal distribution pattern was clear (Fig. 2a), with higher values in summer and lower temperature in winter. But for salinity, seasonal pattern was weak and just appeared in some river estuary region as result of river plume in the wet season. However, the horizontal distribution pattern for sea surface salinity was obviously (Fig. 2b). The higher-lower salinity boundary was almost according with 120°E meridian line.

For the study area, the iso-surface contours of temperature and salinity implied that the hydrological process was rather complex in the Kelantan River Estuary (Figs 3a–d). The warm water at surface was homogeneous, of which the maximum and minimum value of temperature was 31.794°C at Sta. KW15 and 30.248°C at Sta. KW4, respectively. Also, the warm water at bottom layer distributed similarly, of which the maximum and minimum values were 31.840°C at Sta. KW16 and 30.286°C at Sta. KW8, respectively.

On the other hand, the salinity varied even more intense than the temperature did. The maximum and minimum values of salinity at surface water were 32.87 at Sta. KW4 and 12.80 at Sta. KW15, respectively (Fig. 3b). The lower salinity water intruded more dramatically at the bottom water. The maximum and minimum values of salinity at bottom water were 32.50 at Sta. KW7 and 4.00 at Sta. KW14, respectively (Fig. 3d). The salinity was also low at Sta. KW15 at the bottom water, with 8.57 in salinity. The salinity distribution implied that the subterranean estuary or underground water might be the chief source of such low salinity water.

Table 2. The ratio of initial inputting pigment to Chl *a* for the Southern Ocean (Mackey et al., 1996)

	Peri	But-Fuco	Fuco	Hex-Fuco	Neo	Pras	Viol	Allo	Lut	Zea	Chl *b*	Chl *a*
Pras (T3)					0.15	0.32	0.06		0.01		0.95	1.00
Dino	1.06											1.00
Cryp								0.23				1.00
Hapt (T3)				1.70								1.00
Hapt (T4)		0.25	0.59	0.54								1.00
Chlo					0.06		0.06		0.20	0.01	0.26	1.00
Syne										0.35		1.00
Diat			0.75									1.00

Table 3. The ratio of initial inputting pigment to Chl *a* for the South China Sea

	Peri	But-Fuco	Fuco	Hex-Fuco	Neo	Pras	Viol	Allo	Lut	Zea	Chl *b*	Dv-Chl *a*	Chl *a*
Dino	1.06												1.00
Diat			0.75										1.00
Hapt_8		0.25	0.59	0.54									1.00
Hapt_6				1.70									1.00
Chlo					0.06		0.06		0.20	0.01	0.26		1.00
Cryp								0.23					1.00
Proc										0.37	0.68	1.00	
Syne										0.35			1.00
Pras					0.15	0.32	0.06		0.01		0.95		1.00

In the Lembeh Strait, the temperature and salinity varied mildly (Figs 3e, f). The salter water at surface than in the Kelantan River Estuary had the typical characters of the West Pacific Ocean surface water. Maximum values of salinity at surface water were 34.50 at Sta. A13 and 33.90 at the other three Stas A9, A12 and A16, respectively. Although the salinity was higher, the temperature was lower than that in the Kelantan River Estuary significantly. The maximum and minimum values of surface temperature were 27.900°C at Sta. A14 and 28.900°C at Sta. A9, respectively.

The *T*–*S* properties synthetically expressed the characters of

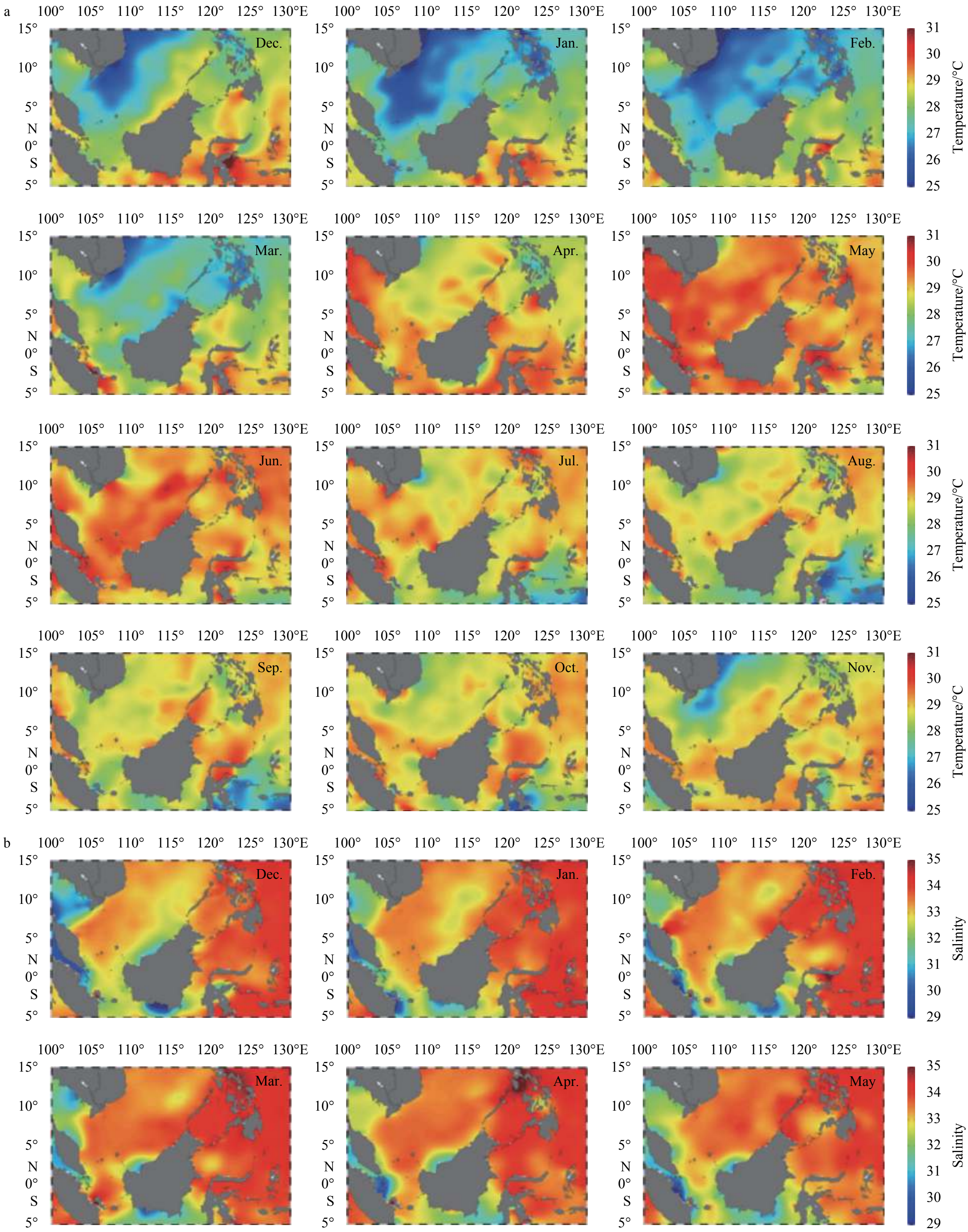

Fig. 2.

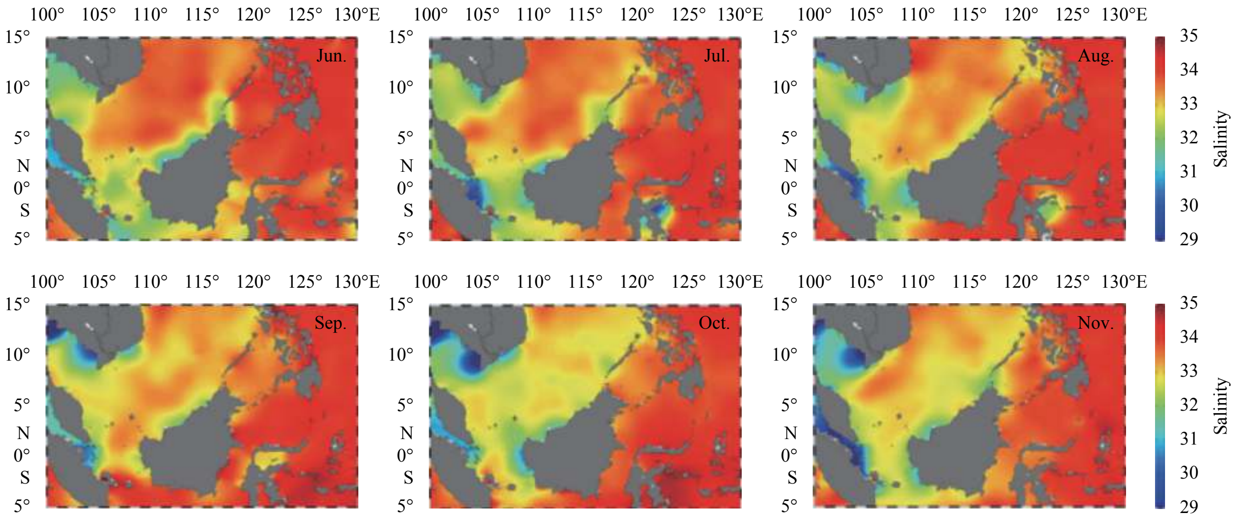

Fig. 2. The distribution of monthly climatology surface temperature (a) and salinity (b) in the southern South China Sea and adjacent area. The data were accessed from the WOA13.

water at the stations both in the Kelantan River Estuary and in the Lembeh Strait (Fig. 4). Less salty and warmer water was obvious in the Kelantan River Estuary along each isopycnal. The potential density (σ_0) was less than 16.0 kg/m^3 at surface water and the bottom low salinity water. In the Lembeh Strait, the σ_0 was more than 20.0 kg/m^3 at surface water, which was similar as the upwelling water from the West Pacific Ocean.

3.2 *Pigments*

The Chl *a* concentration could be a proxy for Chl *a* concentration distribution in the Kelantan River Estuary (Figs 5a, b) and in the Lembeh Strait (Fig. 5c) showed dramatically spatial variation. The Chl *a* concentration was low at surface in the Lembeh Strait, which it was 0.580–0.682 μg/L, with the average (0.620±0.039) μg/L. Nevertheless, the Chl *a* concentration fluctuated violently at surface in the Kelantan River Estuary, in which the biomass was 0.299–3.988 μg/L, with the average (0.922±0.992) μg/L. The biomass at bottom water was higher than at surface in the Kelantan River Estuary, in which the Chl *a* concentration was 0.704–2.352 μg/L, with the average (1.493±0.571) μg/L.

Otherwise, for the other major pigments concentrations, fucoxanthin monopolized pigments in the Kelantan River Estuary (Fig. 6a), but Chl *b*, zeaxanthin and fucoxanthin were three most abundant pigments in the Lembeh Strait (Fig. 6b). In the Kelantan River Estuary, the fucoxanthin/Chl *a* ratio varied 0.045–0.261, the zeaxanthin/Chl *a* ratio varied 0.019–0.168 and the Chl *b*/Chl *a* ratio varied 0.075–0.137 at the surface layer. But at the bottom, the fucoxanthin/Chl *a* ratio varied 0.076–0.301, the zeaxanthin/Chl *a* ratio varied 0.022–0.146 and the Chl *b*/Chl *a* ratio varied 0.050–0.162. It was almost equivalent between the surface and bottom, except a slight higher fucoxanthin/Chl *a* ratio at bottom. In the Lembeh Strait, the fucoxanthin/Chl *a* ratio varied 0.113–0.132, the zeaxanthin/Chl *a* ratio varied 0.081–0.127 and the Chl *b*/Chl *a* ratio varied 0.306–0.333. It showed the very different range of ratio compared to the Kelantan River Estuary, firstly near the mid-value compared with the Kelantan River Estuary approximatively for fucoxanthin/Chl *a*, and secondly the higher ratios for zeaxanthin/Chl *a* and Chl *b*/Chl *a* ratio implied the important contribution by pico-phytoplankton in the Lembeh Strait.

3.3 *Phytoplankton community*

As results of the CHEMTAX, phytoplankton community composition was different in the two study areas (Fig. 7). In the Lembeh Strait, prasinophytes (26.48%±0.83%) and *Synechococcus* (25.73%±4.13%) occupied ~50% of the Chl *a* biomass, followed by diatoms (20.49%±2.34%) and haptophytes T8 (15.13%±2.42%). At surface water in the Kelantan River Estuary, diatoms (58.53%±18.44%) dominated more than half of the phytoplankton biomass, followed by *Synechococcus* (27.27%±14.84%) and prasinophytes (7.00%±4.39%). And it showed the similar status at the bottom water, where diatoms, *Synechococcus* and prasinophytes contributed 64.89%±15.29%, 16.23%±9.98% and 8.91%±2.62%, respectively.

4 Discussion

Although the stratification was remarkable at each station during the cruise, characters on hydrology still varied at different stations. The distinction freshwater fluxes in the Kelantan River Estuary might have impact on the nutrients supplement, following by the patchiness in the phytoplankton biomass and community succession. On the contrary, the upwelling in the coastal region near the Lembeh Strait had the typical characters as the West Pacific water and the oligotrophic status made the regions have lower phytoplankton biomass and the pico-phytoplankton dominated.

Although the low salinity in the Kelantan River Estuary, there was no river plume features during the cruise, and it could be implied that the subterranean estuary or underground water might be the chief source of such low salinity water. The exceptional conditions from this situation could be the poor status in available light. The reduction on water turbidity would be the explanation for the underdevelopment status (Chung et al., 2014; Jiang et al., 2014; Tseng et al., 2014). The turbidity in the Mississippi River Estuary was much lower than that in the Changjiang Estuary, so the biological processes were more active in the former, where the high biomass would appear in plume with 12–20 in salinity compared less biomass below 28 in the Changjiang River Plume (Bianchi et al., 2013). In this study, the decreasing in available light was affected seriously. The warming effect had also been pointed out among inter-annual tendency. An increasing tendency was distinct in the Chl *a* biomass in the Yellow Sea-East

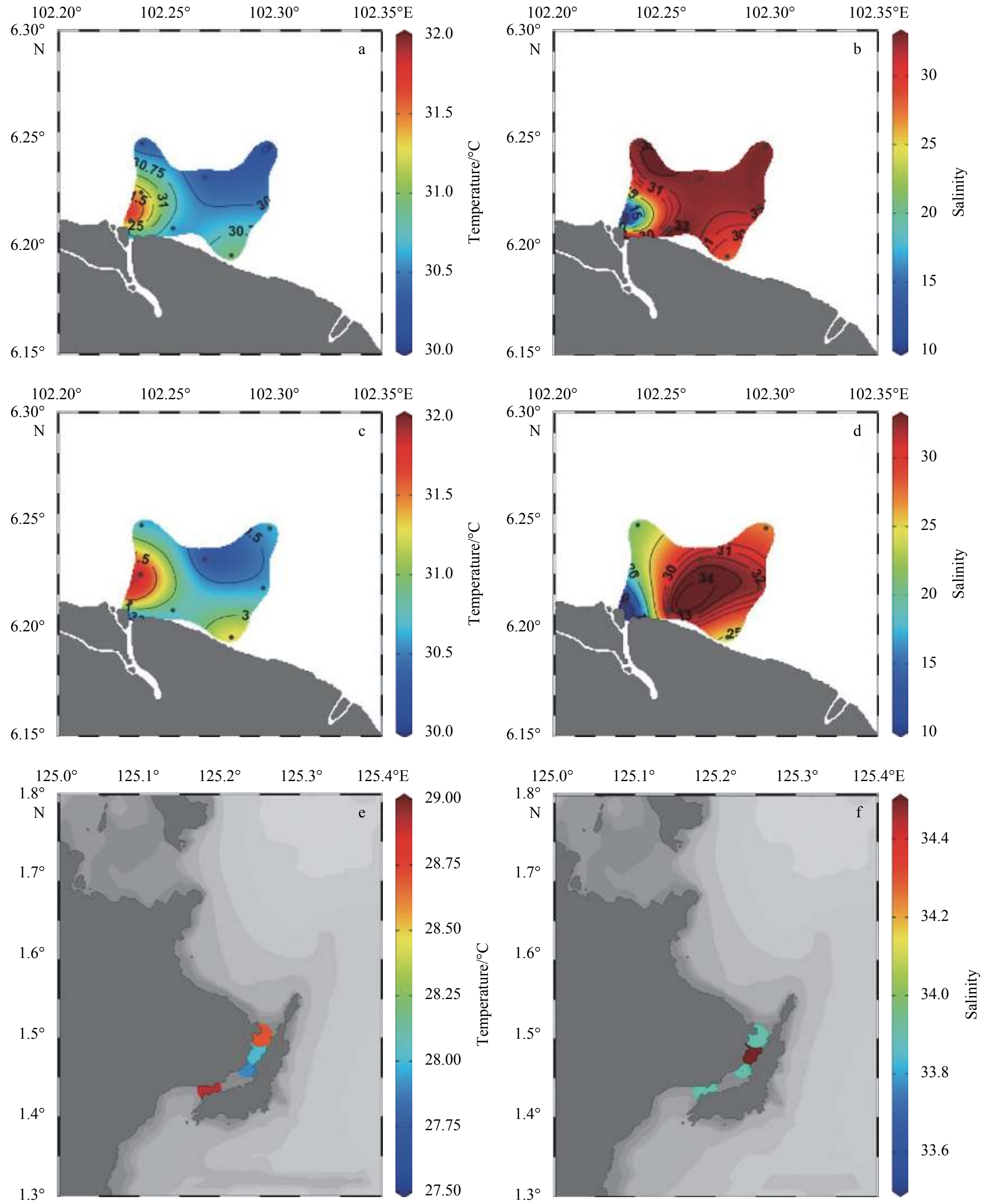

Fig. 3. Temperature and salinity contours at surface (a, b), at bottom (c, d) in the Kelantan River Estuary and at surface in the Lembeh Strait (e, f).

China Sea, both in spring and summer blooms (Xu et al., 2013).

Combining the ratio of pigments to Chl *a* with the salinity distribution could give the information about the adaptation of different phytoplankton groups to the runoff diluted, in general, the terrigenous nutrients supply. In the present study, the ratio of pigments to Chl *a* showed that different pattern neither between the two study areas nor at varied salinity gradients in the Kelantan River Estuary (Fig. 8). The ratioes of fucoxanthin to Chl *a* and zeaxanthin to Chl *a* distributed in mirror image under the salinity neither the riverine nor the salty end-member. It submitted to the normal knowledge that these two groups occupied the different ecological niche, especially in the coastal ecosystem (Brun et al., 2015). For the ratio of Chl *b* to Chl *a*, it was almost steady along the salinity gradient, but was higher in the Lembeh Strait. It could be explained as the prasinophytes with more Chl *b* concentration usually acted as the important component in the oligotrophic West Pacific water (Santos et al., 2017).

Comparison of the phytoplankton community composition was conducted between the two coastal regions (Fig. 9). It could be summarized concisely the more diatoms in the Kelantan River Estuary than in the Lembeh Strait. But the latter possessed more haptophytes T8 and prasinophytes than in Kelantan River Estu-

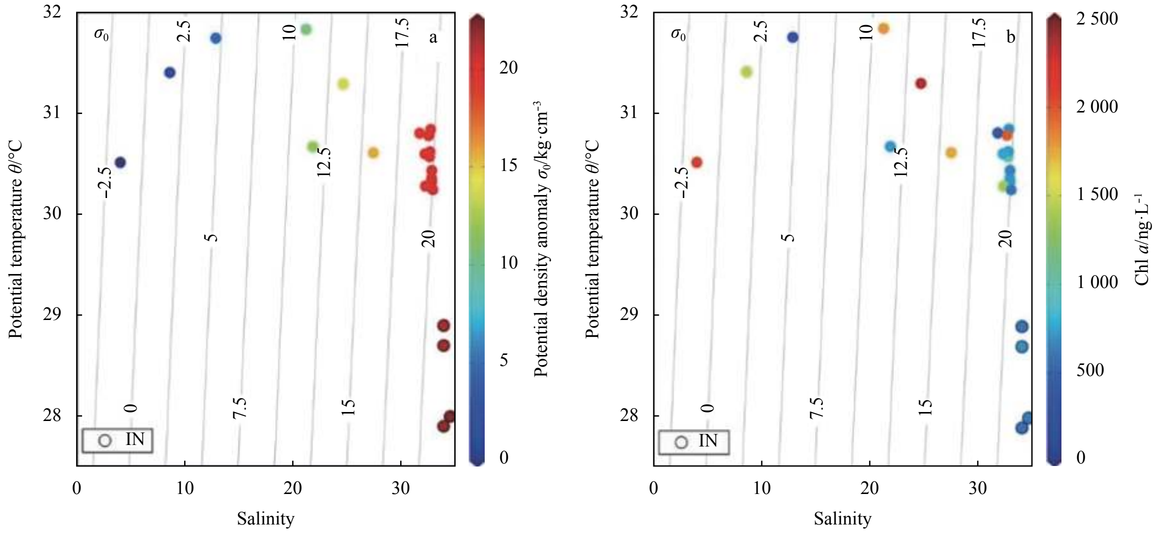

Fig. 4. Potential temperature (θ) versus salinity scatters (*T-S* properties) at the stations. a. The color dots which were seated between the isopycnals (gray lines) implied the potential density anomaly σ_0 (kg/m^3), and b. the Chl *a* concentration (ng/L) standing on the *T-S* properties. IN represents the Lembeh Strait.

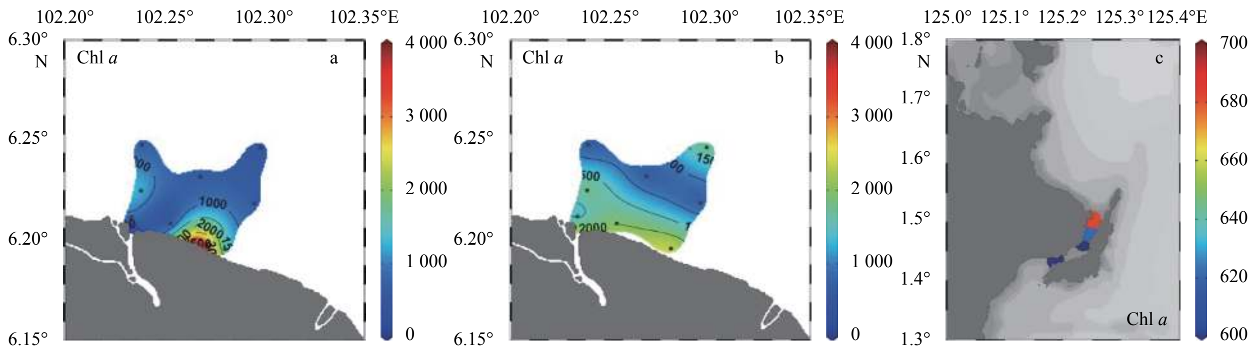

Fig. 5. Chl *a* concentration (ng/L) at surface (a), at bottom (b) in the Kelantan River Estuary and at surface in the Lembeh Strait (c).

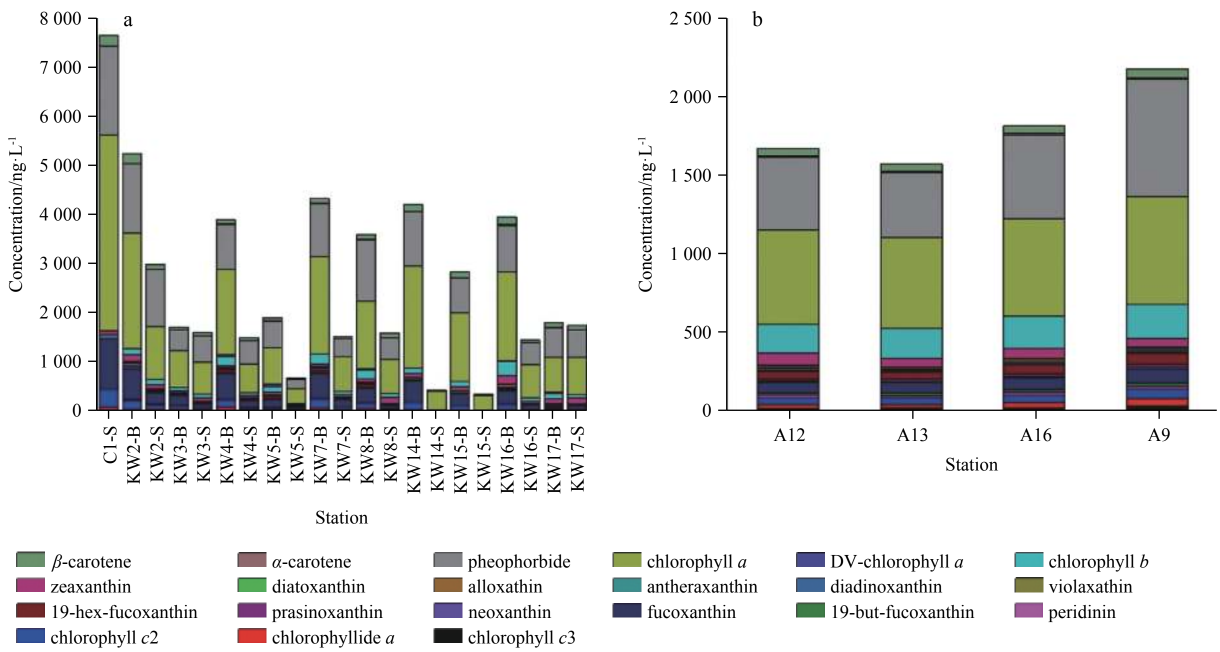

Fig. 6. Pigments concentration and composition in the Kelantan River Estuary (a) and in the Lembeh Strait (b).

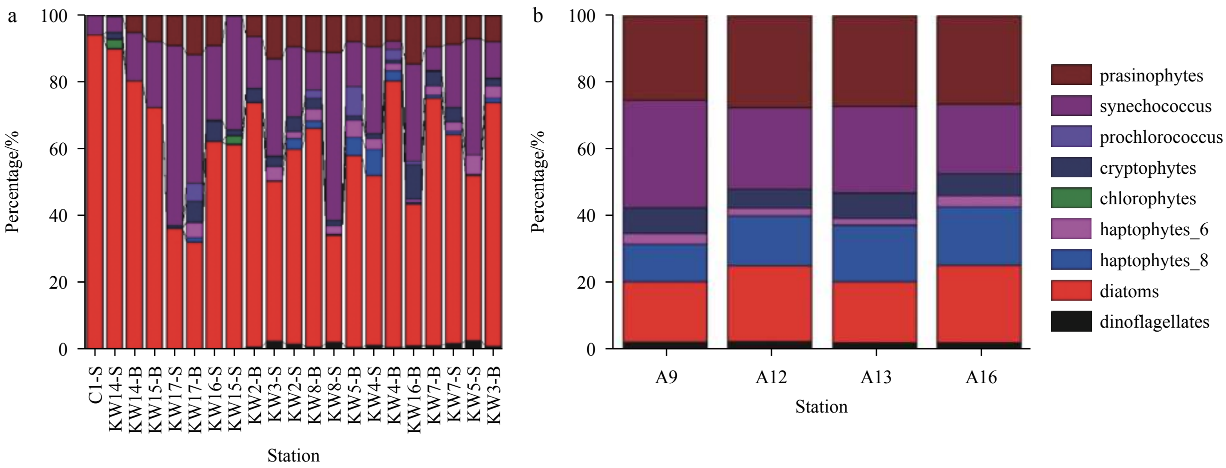

Fig. 7. Phytoplankton community composition in the Kelantan River Estuary (a) and in the Lembeh Strait (b).

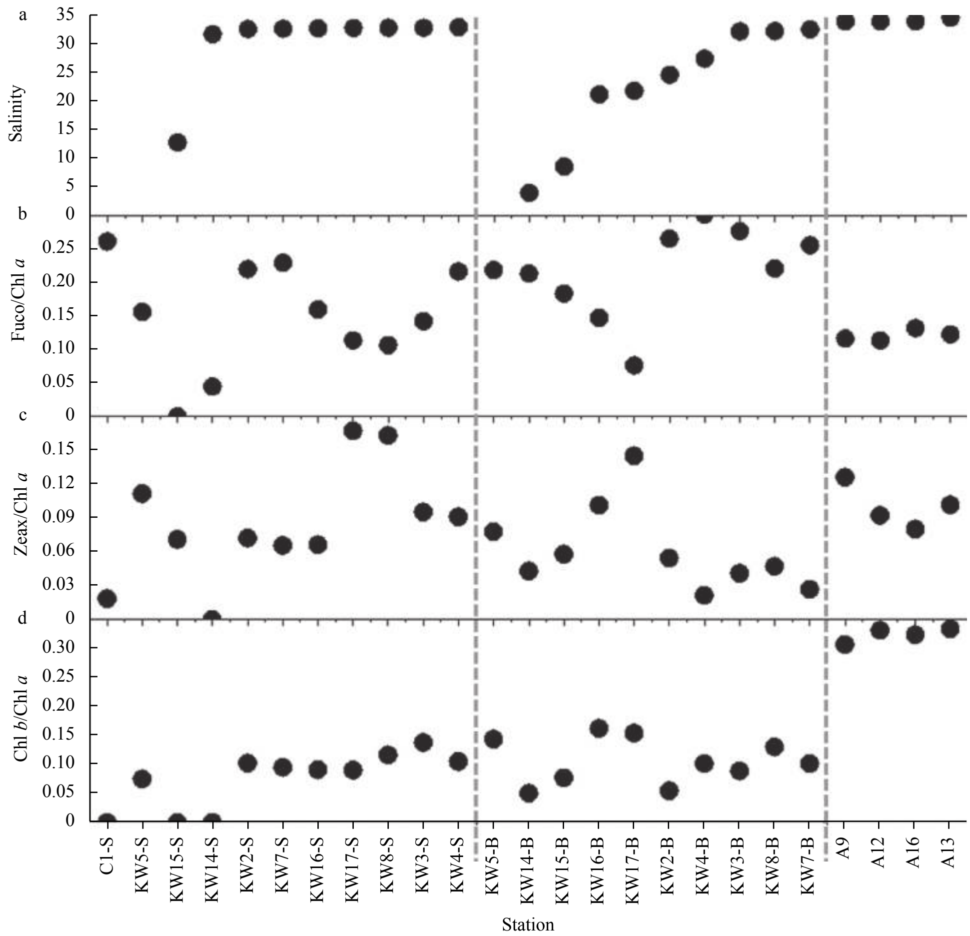

Fig. 8. Salinity (a), fucoxanthin/Chl *a* (b), zeaxanthin/Chl *a* (c) and Chl *b*/Chl *a* (d) ratio at each station.

ary. There was no difference significantly for the *Synechococcus* Chl *a* concentration between the two coastal regions. In our previous study in the Sunda Shelf and the Strait of Malacca, where was adjacent to the Kelantan River Estuary, *Synechococcus* dominated 60%–80% of the total phytoplankton biomass. Diatoms contributed greater than 20% at stations with higher nutrient concentration carrying by the river runoff in the Strait of Malacca (Wang et al., 2015b). The nutrients were the most meaningful entrainment for the biological processes in the riverine discharge. It was obvious that excess nitrate and silicate was in disproportion compared to the Redfield ratio (Redfield, 1958). So it could imply that the different phytoplankton community composition between the two regions implied that the bottom up control affected the phytoplankton biomass in the Lembeh Strait where the oligotrophic water derived from the West Pacific. The terrigenous nutrients supplied the diatoms growing, and pico-phyto-

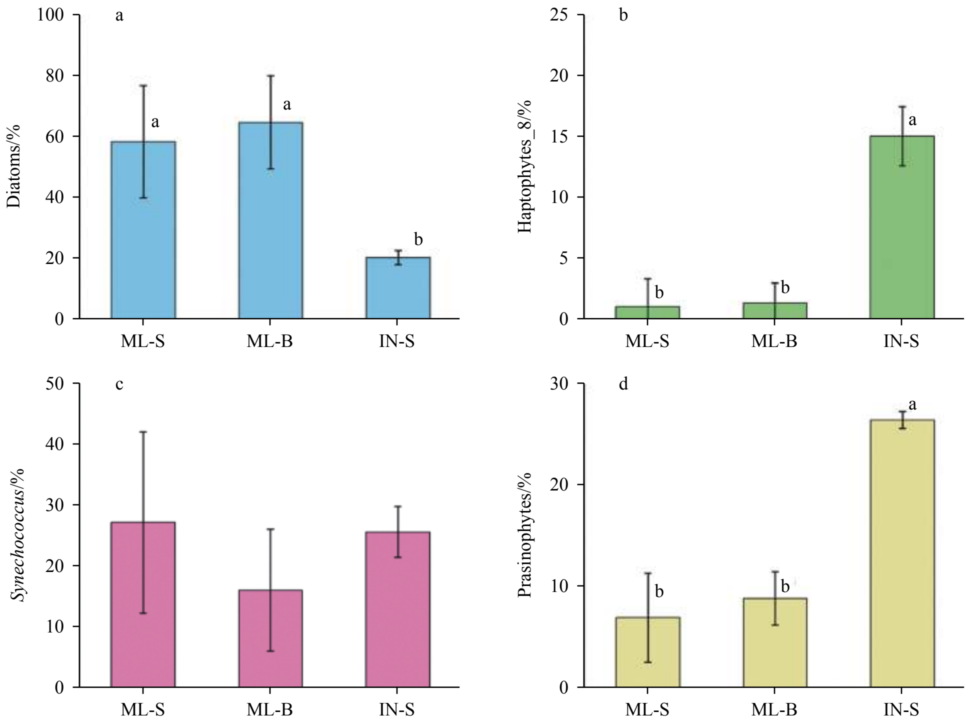

Fig. 9. Percentages of contributors: diatoms (a), Haptophytes_8 (b), *Synechococcus* (c) and prasinophytes (d) to Chl *a* biomass. ML-S represents surface water in the Kelantan River Estuary, ML-B bottom water in the Kelantan River Estuary and IN-S surface water in the Lembeh Strait.

plankton was grazed through top down control in the Kelantan River Estuary.

5 Conclusions

There was significant spatial variation of phytoplankton biomass and community composition in the Kelantan River Estuary and in the Lembeh Strait. The Chl *a* concentration was low at surface in the Lembeh Strait and fluctuated violently at surface in the Kelantan River Estuary. The phytoplankton Chl *a* concentration at bottom water was higher than at surface in the Kelantan River Estuary. Pico-phytoplankton, especially the *Synechococcus* and prasinophytes were abundant in the Lembeh Strait, but diatoms dominated in the Kelantan River Estuary. The different phytoplankton community composition between the two regions implied that the bottom up control affected the phytoplankton biomass in the Lembeh Strait. The terrigenous nutrients supplied the diatoms growing, and pico-phytoplankton was grazed through top down control in the Kelantan River Estuary.

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