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Effects of tidal events on the composition and distribution of phytoplankton in Merbok river estuary Kedah, Malaysia

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Abstract: The impact of tidal events on water quality and on the species composition and distribution of phytoplankton at three stations along Merbok river estuary, Malaysia were investigated. Bacillariophyta was the most dominant group, followed by, in decreasing order, Dinophyta, Chlorophyta, Euglenophyta, Cyanophyta and Cryptophyta. Spring tides had higher total cell number and richness but neap tides had higher diversity (except Station 2) and evenness of phytoplankton at all stations. Spring tides also led to higher salinity, conductivity, total suspended solids, nitrate and orthophosphate but lower pH, dissolved oxygen, transparency, chlorophyll-*a* and nitrite. No significant effects of tidal changes were observed on temperature, light intensity and ammonia. *Oscillatoria* spp. was dominant at Station 1, while *Cylindrotheca* spp. and *Cyclotella* spp. occurred at all stations and were dominant at Stations 2 and 3, respectively. Higher abundance of phytoplankton at Station 1 corresponded to significantly higher nitrate and ammonia but lower temperature, pH, dissolved oxygen, salinity, conductivity, transparency, total suspended solids, nitrite and orthophosphate. Water quality varied according to the tidal events, which subsequently affected the composition and distribution of phytoplankton, thus reflecting the importance of biotic and abiotic parameters in understanding the overall ecological status of the Merbok river estuary.

Key words: Chlorophyll-*a*, microalgae, neap tides, spring tides, water quality.

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Introduction

Estuaries are some of the most productive systems (Pereira-Filho *et al*. 2001) responsible for the habitat, nursery, feeding, breeding and protective grounds for various animal species such as fish, molluscs, crustaceans, birds and mammals (Ohrel & Register 2006). Estuaries also support fisheries, transportation and recreational activities, and they are an excellent natural buffer for protecting uplands from storms and waves as well as for filtering excess nutrient and pollution (Gao & Song 2005).

Estuaries are normally located at the river mouths that lead to the sea (Elliott & McLusky 2002). Hence, estuaries receive continuous mixed inflow from both sea water and fresh water aided by wind movement. Seawater inflow is influenced by the tidal changes of spring tides and neap tides while fresh water inflow is contributed by nearby

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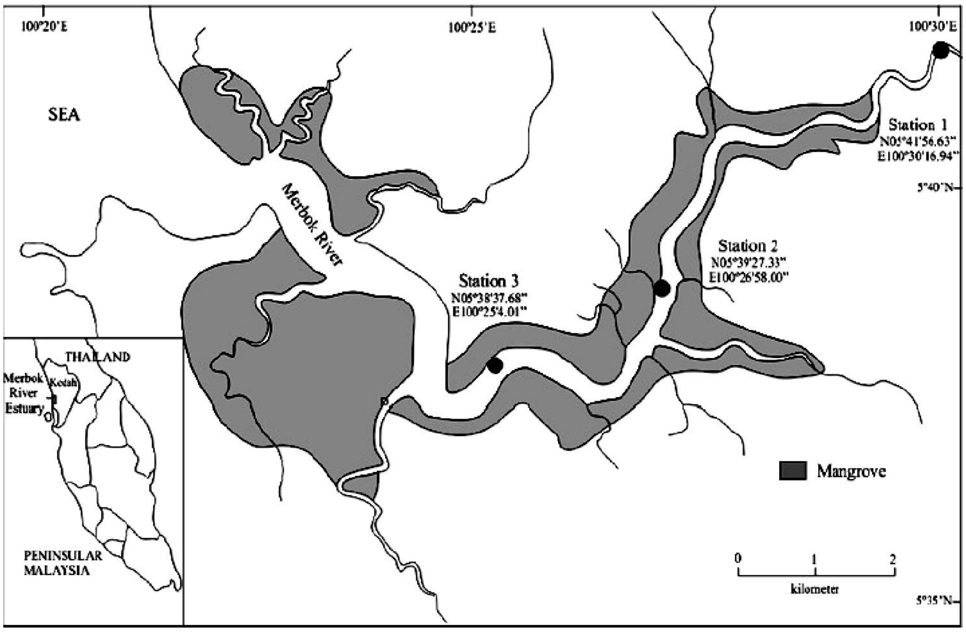


Fig. 1. Map and sampling stations along the Merbok River estuary. Shaded areas indicate the location of mangroves.

rivers (Priya *et al.* 2012). The changes of water levels and turbulences in estuaries during spring-neap tides as well as monsoonal season would affect water quality such as total suspended solids (Chen *et al.* 2006; Spellman 2011), dissolved oxygen (Perkins 1974), water temperature (Olausson & Cato 1980), salinity (Ohrel & Register 2006; Prasanna & Ranjan 2010), pH (Spellman 2011), conductivity (Smith 1992), light intensity (Dennison *et al.* 1993), transparency (Borja & Collins 2004; Wangersky 2006), surface and bottom currents (Kramer *et al.* 1994), nitrogen (Kennish 2002; Neil 2005), phosphate (Vander Zee *et al.* 2007) and chlorophyll-*a* concentrations (Conley *et al.* 2000; Zheng *et al.* 2004) which would influence the growth, biomass and species composition of phytoplankton (Aquino *et al.* 2015; Canini *et al.* 2013; Domingues *et al.* 2010; Lauria *et al.* 1999).

Composition and distribution of phytoplankton are essential factors in estuaries as phytoplankton are the primary food producers affecting the population of other organisms along the food chain (Lehman 2007). The biomass of phytoplankton and blooms of certain species such as the high pollution tolerant diatoms (Bere 2014; Wan Maznah & Mansor 2002), toxin producing cyanobacteria and dinoflagellates (Kirkpatrick *et al.* 2004) could affect the economy in terms of water quality deterioration and mortality of commercial fish species (Cloern 1991). Microalgae have rapid regeneration rates, very short life cycles and high sensitivity towards environmental changes which make them suitable for the biological monitoring degree of impact on the ecological systems (Wan Maznah 2010). In Malaysia, the expansion of urbanization, industrialization and agriculture activities have limited the quantity and quality of water supply. Out of 143 river basins in Malaysia, clean rivers have dropped from 91 to 76, thus increasing the slightly polluted river from 45 to 60 in 2009 while 7 rivers remained polluted as compared with the year 2007 (DOE 2009). Therefore, it is of great importance that water qualities are closely monitored in relation to different composition and distribution of phytoplankton in order to better understand the impacts of human activities on any aquatic ecosystem. For instance, organic and inorganic chemicals that flow into the rivers could affect the organisms and might induce changes to the phytoplankton composition (Round 1991).

Merbok river is a mangrove estuary described as a semidiurnal mesotidal system, with range of spring and neap tides of 2.3 and 0.8 m, respectively (Ong *et al.* 1991). The spring-neap tides variations are affecting the current and salinity stratification in Merbok river estuary (Uncles *et al.* 1990). The estuary comprised an area of about 8000 hectares with 5000 hectares of

Table 1. Sampling dates, time, tides and range of tidal heights during the sampling period.

Dates	Time	Tides	Range of tidal heights (m)
12 Nov 2011	0900 - 1100	Spring tide	0.9 - 2.9
13 Nov 2011	1500 - 1700	Spring tide	1.5 - 4.5
13 Nov 2011	2100 - 2300	Spring tide	0.9 - 1.5
13 Nov 2011	0300 - 0500	Spring tide	0.5 - 2.5
19 Nov 2011	0900 - 1100	Neap tide	1.0 - 3.0
19 Nov 2011	1500 - 1700	Neap tide	1.0 - 3.5
19 Nov 2011	2100 - 2300	Neap tide	0.8 - 4.0
20 Nov 2011	0300 - 0500	Neap tide	1.5 - 3.5
26 Nov 2011	0900 - 1100	Spring tide	1.0 - 4.5
26 Nov 2011	1500 - 1700	Spring tide	1.0 - 3.5
26 Nov 2011	2100 - 2300	Spring tide	0.8 - 2.5
27 Nov 2011	0300 - 0500	Spring tide	0.5 - 1.5
3 Dec 2011	0900 - 1100	Neap tide	0.5 - 2.9
3 Dec 2011	1500 - 1700	Neap tide	1.0 - 4.0
3 Dec 2011	2100 - 2300	Neap tide	1.0 - 3.0
4 Dec 2011	0300 - 0500	Neap tide	1.2 - 3.5

mangrove forest reserve, 1500 hectares of reclaimed mangrove and 1500 hectares of water ways (Ong *et al.* 1991). Approximately 975 hectares were transformed into aquaculture ponds including floating cage cultures, small industries and various development projects (Jusoff 2008). Merbok river estuary receives inflow of seawater from Straits of Malacca as well as fresh water from nearby streams and land runoffs. Therefore, the ecological health and influence of tidal changes on the distribution and composition of its phytoplankton are of great concern. This study was conducted to identify the composition and distribution of phytoplankton species during the spring-neap tides and to relate it with the water quality parameters at three selected stations along the Merbok river estuary.

Materials and methods

Sampling site

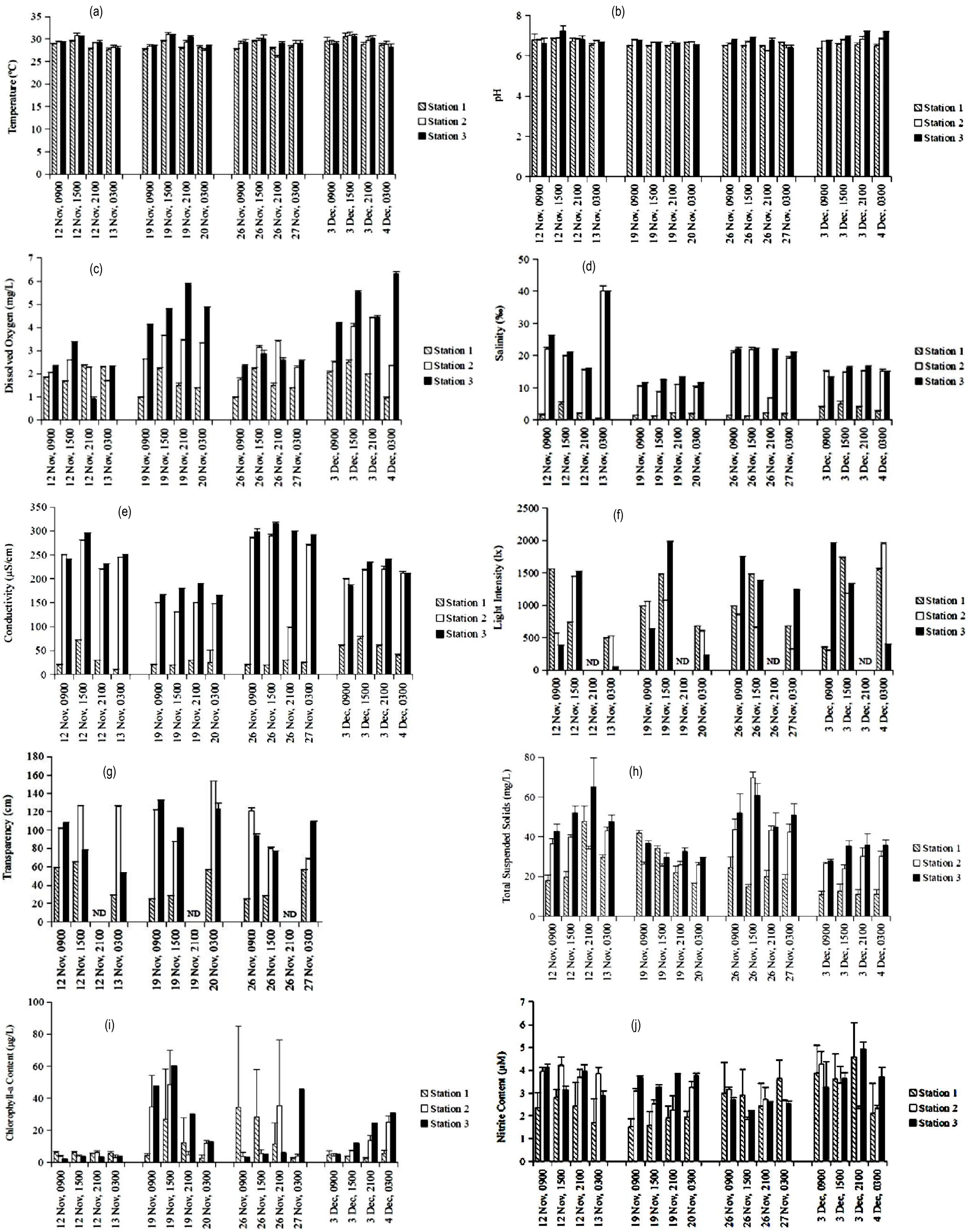
Merbok river estuary was located in southern Kedah at 05° 30' N and 100° 25' E in northwest Peninsular Malaysia (Ong *et al.* 1991) (Fig. 1). It lays between the foot of Gunung Jerai Forest Reserve to the north and Sungai Muda to the south, and between the town of Sungai Petani to the east and coastal area to the west (Abdullah 2011). The length of Merbok river was about 35 km with most part being estuarine except a few kilometres of fresh water in the upstream (Ong *et al.* 1991). The river had a width of 20 m in the upper reaches and 2 km at the mouth and the depth varies between 3 and 15 m (Ong *et al.* 1991). The seawater flowed up the estuary to about 30 km while the fresh water enters the estuary from ground runoff and from several small streams flowing into tributaries of the estuary (Ong *et al.* 1991).

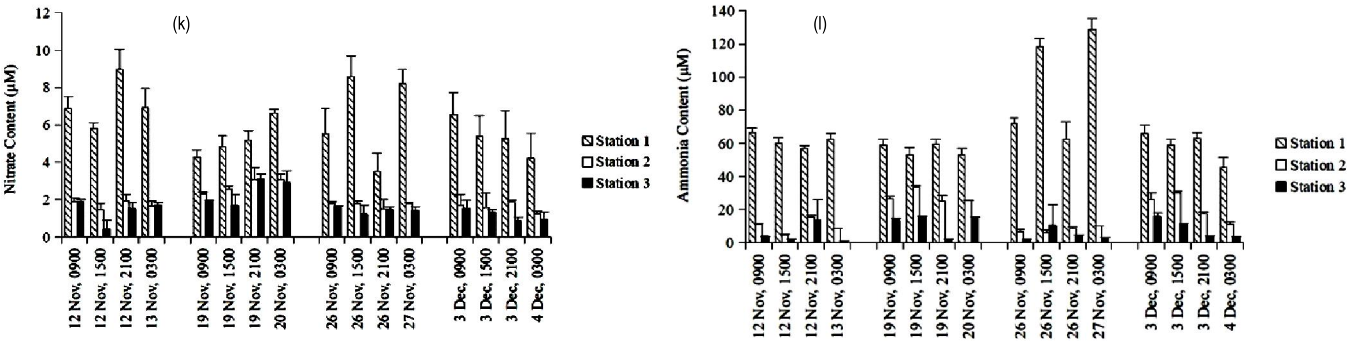
Merbok river was dry and warm from January to April and wet from May to December. The temperature averaged between 21 and 32 ºC with annual rainfall between 2000 and 2500 mm (Abdullah 2011). It had a tropical monsoon climate with the northeastern monsoon occuring between November and March while the southwestern monsoon occurs between May to September (Ong *et al.* 1991).

Sampling design

Phytoplankton composition and distribution as well as the water quality parameters were determined in three sampling stations along Merbok river estuary (Fig. 1). Station 1 was located at the Lalang river in the upstream of Merbok river at 05° 41' 56.63” N 100° 30' 16.94” E. Lalang river is affected by residential area. Station 2 on the Jagong river and Station 3 on the Gelam river were in the mid stream and downstream of Merbok river, respectively. Stations 2 and 3, situated at 05° 39' 27.33” N 100° 26' 58.00” E and 05° 38' 37.68” N 100° 25' 4.01” E respectively are impacted by aquaculture activities. Sampling was conducted weekly with alternate spring and neap tides, for 24 hours at 6-hour intervals from 12th Nov 2011 to 4th Dec 2011 as presented in Table 1.

During sampling events, water qualities were


(a)
Temperature (°C)
(b)
pH
(c)
Dissolved Oxygen (mg/L)
(d)
Salinity (‰)
(e)
Conductivity (µS/cm)
(f)
Light Intensity (lx)
(g)
Transparency (cm)
(h)
Total Suspended Solids (mg/L)
(i)
Chlorophyll-a Content (µg/L)
(j)
Nitrite Content (µM)
ND
▨ Station 1
☐ Station 2
■ Station 3
12 Nov, 0900
12 Nov, 1500
12 Nov, 2100
13 Nov, 0300
19 Nov, 0900
19 Nov, 1500
19 Nov, 2100
20 Nov, 0300
26 Nov, 0900
26 Nov, 1500
26 Nov, 2100
27 Nov, 0300
3 Dec, 0900
3 Dec, 1500
3 Dec, 2100
4 Dec, 0300



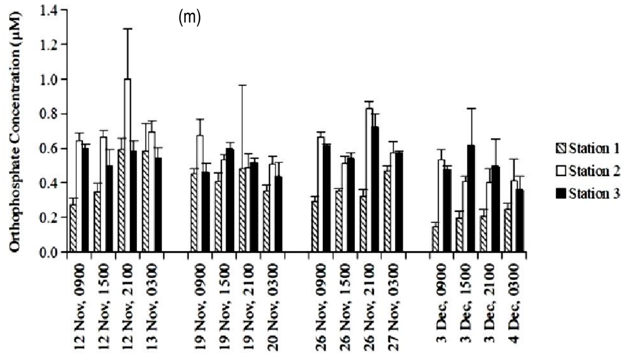


Fig. 2. Temporal water quality parameters for spring and neap tides at Stations 1, 2 and 3 during sampling period. (a) temperature (°C); (b) pH; (c) dissolved oxygen (mg L^{-1}); (d) salinity (‰); (e) conductivity (µS cm^{-1}); (f) light intensity (lx); (g) transparency (cm); (h) total suspended solids (mg L^{-1}); (i) chlorophyll-*a* (µg L^{-1}); (j) nitrite (µM); (k) nitrate (µM); (l) ammonia (µM); (m) orthophosphate (µM).

measured *in situ*. Water temperature and dissolved oxygen were measured using dissolved oxygen meter (YSI, Mode l52) while pH was determined using Smartest TM series (Model pH Scan 2). Salinity and conductivity were measured using SCT meter (Model YS I22). Light intensity was measured using Data Logging Light Meter (RS-232) and water transparency was measured using Secchi disk. Measurements of total suspended solids (TSS), chlorophyll-*a,* nitrite, nitrate, ammonia and orthophosphate were conducted according to the methods described by Adams (1990) and Strickland & Parsons (1972). Surface water was collected at each station and poured into pre-cleaned polyethylene bottles, capped and labelled. The bottles were kept in an ice chest box during transportation to the laboratory and kept in a freezer at -20 ºC upon arrival prior to analysis.

Phytoplankton composition and community structure analysis

To collect phytoplankton samples, 40 L of surface water were collected using 5 L bucket and poured into a plankton net (Wildco® Instruments) with a mesh size of 35 µm. A quantity of 100 mL filtrate which contains phytoplankton were preserved in a labelled bottle with an addition of 1 mL Lugol's solution. Triplicate samples were collected for each station. Phytoplankton samples were identified under the light microscope (Nikon, Model YS 100) with a cover slip (22 mm x 22 mm), magnified at 10 x, 20 x, 40 x and 100 x with the help of taxonomic keys, drawings and descriptions given in Sournia (1978), Tomas (1997), Ahmed (2009), Pentecost (1984), Round *et al.* (1990) and Smith & Johnson (1996). Phytoplankton composition and enumeration was determined according to Lobban *et al.* (1988). Specimens could only be identified to genus level based on the available literature, so in this study, the phytoplankton community structural analyses will be based on the genus as taxonomic units (Heip *et al.* 1998). Relative abundance of each phytoplankton genus was calculated according to APHA (1998) based on the ratio of abundance of each phytoplankton genus from the total abundance of all phytoplankton genera, and presented in percentages.

Dominant phytoplankton genera at all sampling stations were determined using the Importance Species Index (ISI), modified from Rushforth & Brock (1991) as: ISI = f_ix D_i, where, f_i is the relative frequency of genus i and D_i is the average relative density of genus i.

This index is preferable because it reflects both the distribution and abundance of a taxon in the ecosystem (Wan Maznah & Mansor 2000). Richness and Shannon-Wiener (H’) indices were calculated according to Ludwig & Reynolds (1998). as: H' = Σ (Ni/N) $\log_2$ (Ni/N), where, (Ni / N) is the probability of getting genus *i* in a sample; N*i* is the number of individuals of genus *i* and N is the total number of individuals of all genus. Evenness index (E) was conducted to measure how evenly the individuals in the community are distributed among the different genus (Heip *et al.* 1998), following Ludwig & Reynolds (1998) as: E = H' ln S, where, H' is the diversity index and S is the number of genus.

Statistical analysis

All data were analyzed using SPSS Statistics Version 20 (SPSS, Chicago, IL, USA). Phytoplankton community structural analysis was determined using Multivariate Statistical Program (MVSP) Version 3.13 d (Kovach Computing Services, UK). One way ANOVA was used to detect statistically significant differences in environmental parameters and abundance of phytoplankton between sampling stations. All data were analyzed for normality using a Shapiro-Wilk normality test prior to analysis. Correlation between the densities of frequently occurring phytoplankton genus with ISI value higher than 1.00 with water quality parameters were identified using Pearson's Correlation.

Results

Water quality

Temporal water quality parameters during the sampling period for alternate spring and neap tides are presented in Figs. 2 a-m. Temperature ranged between 26.00 ± 0.44 - 30.80 ± 0.44 °C and 27.50 ± 0.44 - 31.10 ± 0.36 °C (Fig. 2a) while pH were between 6.14 ± 0.54 - 7.20 ± 0.26 and 6.35 ± 0.04 - 7.17 ± 0.06 (Fig. 2b) for spring tides and neap tides respectively. Dissolved oxygen was generally lower during spring tides ranging from 0.90 ± 0.10 - 3.94 ± 0.06 mg L^{-1} compared to neap tides 0.94 ± 0.04 - 6.31 ± 0.09 mg L^{-1}. Overall, dissolved oxygen was lowest at Station 1, followed by Station 2 and highest at Station 3 (Fig. 2c). Salinity and conductivity (Figs. 2d & 2e, respectively) were also lower at Station 1, followed by Stations 2 and 3 with spring tides having higher values than neap tides. Light intensity ranged between 47.00 ± 3.50 - 1811.00 ± 10.10 lx and 230.0 ± 3.5 - 1983 ± 2.6 lx (Fig. 2f) while transparency was between 29.00 ± 0.17 - 126.50 ± 0.20 cm and 25.50 ± 0.17 - 154.00 ± 0.10 cm for spring and neap tides respectively (Fig. 2g). Light intensity and transparency were not detected for all sampling period between 21:00 - 23:00 during the night (Figs. 2f & 2g). Total suspended solids were generally higher during spring tides especially at Station 3 (Fig. 2h). Chlorophyll-*a* content showed a wide range of results between 2.26 ± 0.69 - 45.49 ± 44.63 μg L^{-1} for spring tides sand between 2.27 ± 0.81 - 59.96 ± 1.93 μg L^{-1} for neap tides (Fig. 2i). During spring tides, nitrite content ranges between 1.70 ± 0.001 - 4.22 ± 0.007 μM, while during neap tides it ranges between 1.52 ± 0.004 - 4.92 ± 0.015 μM (Fig. 2j). In general, nitrate (Fig. 2k) and ammonia (Fig. 2l) were higher at Station 1 compared to Stations 2 and 3 while orthophosphate (Fig. 2m) was lower at Station 1 than Stations 2 and 3.

Water quality parameters for Stations 1, 2 and 3 for both spring and neap tides are presented in Table 2. During spring tides, the range of tidal heights were between 0.5 and 4.5 m while during neap tides, the range of tidal height were between 0.5 and 4.0 m (Table 1). Significant differences were observed for salinity ($P < 0.000$), conductivity ($P < 0.000$), total suspended solids ($P < 0.000$), nitrate ($P < 0.000$) and ortho phosphate ($P < 0.000$)), pH ($P < 0.024$), dissolved oxygen ($P < 0.000$), transparency ($P < 0.000$), chlorophyll-*a* ($P < 0.041$) and ammonia ($P < 0.000$). There were no significant differences observed for temperature ($P > 0.384$), light intensity ($P > 0.870$) and nitrite ($P > 0.064$).

Phytoplankton composition and community structure

In the present study, 56 genera of phytoplankton from 6 groups were identified along Merbok river estuary where 51 genera were present during spring tides and 41 genera were found during neap tides. In total, Bacillariophyta was dominant followed by Dinophyta, Chlorophyta, Euglenophyta, Cyanophyta and Cryptophyta. Generally, spring tides showed higher number of genera at Stations 1 (36 genera), 2 (33 genera) and 3 (37 genera) compared to neap tides (Table 3). Relative abundance of phytoplankton groups at each station are illustrated in Fig. 3. Bacillariophyta dominated the phytoplankton population at Stations 1, 2 and 3 with 60.7, 61.3 and 59.4 %, respectively. The second largest phytoplankton population at Station 1 was Chlorophyta with 14.8 %, and Dinophyta was the second largest phytoplankton population at Stations 2 and 3 with 16.1 and 17.2 %, respectively. The smallest phytoplankton population were

Table 2. Water quality parameters at Stations 1, 2 and 3 during spring and neap tides.

Water quality parameters	Spring tides			Neap tides		
	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3
Temperature (°C)	28.40 ± 0.78	28.90 ± 1.39	29.24 ± 0.74	28.85 ± 0.94	29.30 ± 1.21	29.48 ± 1.14
pH	6.62 ± 0.15	6.63 ± 0.22	6.75 ± 0.23	6.50 ± 0.08	6.73 ± 0.09	6.81 ± 0.25
Dissolved oxygen (mg L^{-1})	1.78 ± 0.49	2.39 ± 0.61	2.40 ± 0.70	1.70 ± 0.58	3.29 ± 0.75	5.01 ± 0.81
Salinity (‰)	1.96 ± 1.33	15.76 ± 8.11	18.69 ± 8.02	2.81 ± 1.38	12.59 ± 2.71	13.65 ± 1.99
Conductivity (µS cm^{-1})	28.38 ± 18.78	242.25 ± 62.88	277.00 ± 31.91	41.29 ± 21.14	178.50 ± 3710	195.88 ± 28.69
Light intensity (lx)	990.67 ± 400.08	728.83 ± 391.73	1050.17 ± 678.97	1132.00 ± 547.67	1030.33 ± 564.81	1090.17 ± 781.11
Transparency (cm)	44.08 ± 18.24	103.92 ± 25.12	86.50 ± 21.50	58.67 ± 31.69	119.08 ± 24.36	128.75 ± 14.65
Total suspended solids (mg L^{-1})	24.08 ± 10.56	44.00 ± 10.99	51.87 ± 7.69	19.93 ± 11.86	26.78 ± 2.30	32.72 ± 3.49
Chlorophyll-*a* (µg L^{-1})	12.40 ± 12.00	8.23 ± 11.06	9.081 ± 4.76	7.79 ± 8.36	18.72 ± 16.11	27.80 ± 18.75
Nitrite (µM)	2.66 ± 0.57	3.26 ± 0.81	3.00 ± 0.70	2.64 ± 1.20	2.93 ± 0.71	3.75 ± 0.52
Nitrate (µM)	6.79 ± 1.83	1.72 ± 0.17	1.39 ± 0.44	8.28 ± 0.89	2.18 ± 0.69	1.76 ± 0.83
Ammonia (µM)	78.37 ± 2840	8.88 ± 3.44	4.74 ± 4.46	57.15 ± 6.48	24.40 ± 7.01	9.81 ± 5.97
Orthophosphate (µM)	0.40 ± 0.13	0.70 ± 0.15	0.58 ± 0.07	0.31 ± 0.13	0.49 ± 0.09	0.49 ± 0.08

Values are mean ± SD.

Table 3. Number of genus for each group of phytoplankton at Stations 1, 2 and 3 during spring and neap tides.

Group	Spring tides			Neap tides		
	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3
Bacillariophyta	22	20	25	15	18	13
Dinophyta	2	5	6	0	5	5
Chlorophyta	5	2	1	4	1	3
Euglenophyta	4	3	1	3	2	2
Cyanophyta	2	2	3	2	2	3
Cryptophyta	1	1	1	1	1	1
Total	36	33	37	25	29	27

Cryptophyta and Dinophyta both at 3.3 % at Station 1, while the least phytoplankton population was Cryptophyta at Stations 2 and 3 with 3.2 and 3.1 %, respectively.

During spring tides, the total abundance of phytoplankton was highest at Station 1 followed by Stations 2 and 3 at 8.18 X 10^7, 7.26 X 10^7 and 5.69 X 10^7 cells m^{-3}, respectively. On the other hand, during neap tides, the total abundance of phytoplankton was highest at Station 2 (7.43 X 10^7 cells m^3), followed by Stations 1 (6.45 X 10^7 cells m^{-3}) and 3 (6.47 X 10^7 cells m^{-3}) (Fig. 4).

The relative abundance of identified phytoplankton genus at Stations 1, 2 and 3 during both spring and neap tides are presented in Table 4. *Chaetoceros* spp., *Coscinodiscus* spp., *Cyclotella* spp., *Cylindrotheca* spp., *Navicula* spp., *Nitzschia* spp., *Gyrosigma* spp., *Skeletonema* spp., *Trachelomonas* spp., *Cryptomonas* spp. and *Oscillatoria* spp. were present both during spring and neap tides at all stations and *Coscinodiscus* spp., *Cyclotella* spp., *Cylindrotheca* spp., *Navicula* spp., *Nitzschia* spp. and *Oscillatoria* spp. showed more than 1 % of relative abundance regardless of tidal events and stations. *Achnanthes* spp., *Bacteriastrum* spp., *Climacodium* spp., *Guinardia* spp., *Heterothrix* spp., *Pseudonitzschia* spp., *Rhizosolenia* spp., *Thalassionema* spp., *Triceratium* spp., *Alexandrium* spp., *Ceratium* spp., *Pentapharsodinium* spp., *Ophiocytium* spp. were not found during neap tides at all stations while *Mallomonas* spp., *Lingulodinium* spp. and *Anabaena* spp. were not found during spring tides at all stations.

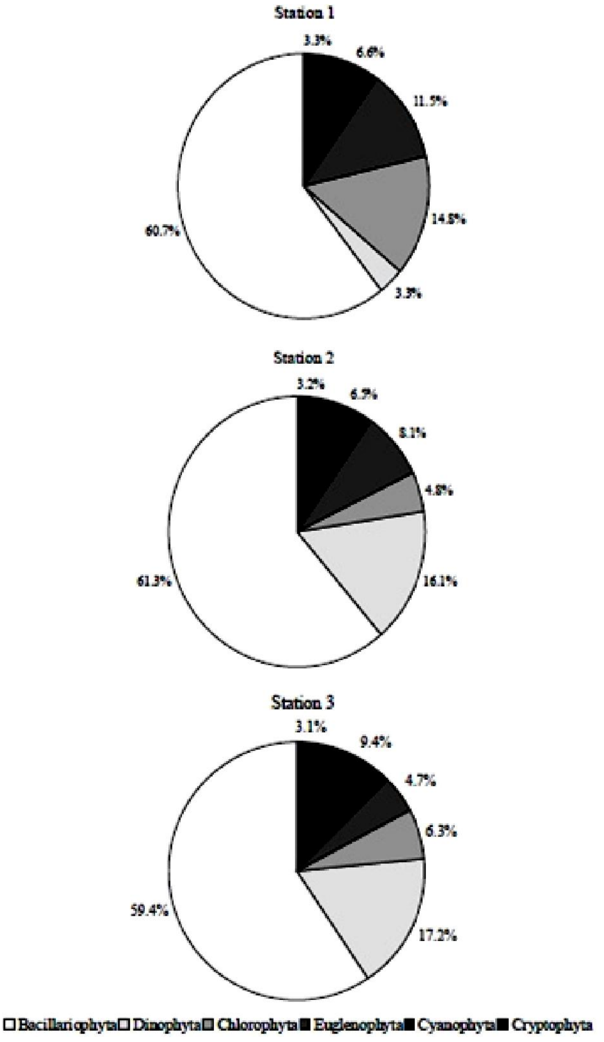


Fig. 3. Percent relative abundance of phytoplankton groups at Stations 1, 2 and 3.

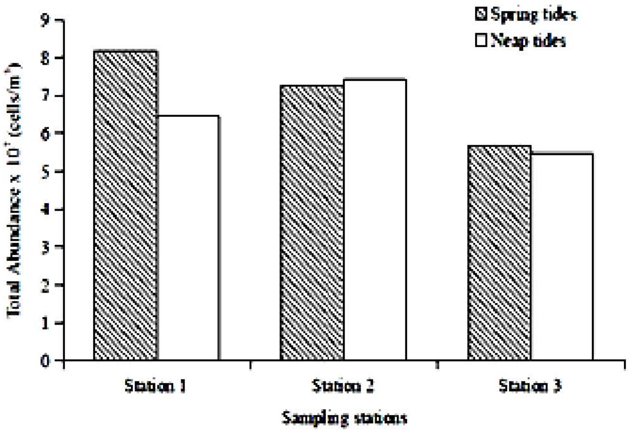


Fig. 4. Total abundance of phytoplankton for spring and neap tides at Stations 1, 2 and 3.

Importance Species Index (ISI) (at genus level) revealed 9 dominant phytoplankton genera with ISI higher than 1.00 depending on stations (Table 5). *Cylindrotheca* spp. (ISI = 18.21), *Nitzschia* spp. (ISI = 6.39), *Cyclotella* spp. (ISI = 5.25), *Oscillatoria* spp. (ISI = 4.08), *Navicula* spp. (ISI = 1.45), *Gyrosigma* spp. (ISI = 1.39) and *Chaetoceros* spp. (ISI = 1.30) were dominant at Station 2, while *Oscillatoria* spp. (ISI = 28.82), *Cyclotella* spp. (ISI = 27.54), *Cryptomonas* spp. (ISI = 6.72), *Cylindrotheca* spp. (ISI = 4.05) and *Navicula* spp. (ISI = 2.55) dominated the Station 1. At Station 3, *Cyclotella* spp. (ISI = 12.47), *Cylindrotheca* spp. (ISI = 8.52), *Coscinodiscus* spp. (ISI = 2.93), and *Chaetoceros* spp. (ISI = 1.99) were dominant. The most dominant genus at Stations 1, 2 and 3 were *Oscillatoria* spp. (ISI = 28.82), *Cylindrotheca* spp. (ISI = 18.21) and *Cyclotella* spp. (ISI = 12.47), respectively while *Cyclotella* spp. and *Cylindrotheca* spp. were abundant and frequently occurred at all sampling stations.

Richness, Shannon-Wiener's diversity (H') and evenness (E) indices for Stations 1, 2 and 3 during spring and neap tides are presented in Table 6. Spring tides recorded highest and lowest richness index of phytoplankton at Stations 3 and 2 with 37 and 33 genera, respectively. On the other hand, neap tides revealed the highest and lowest richness index of phytoplankton at Stations 2 and 1, with 29 and 25 genera, respectively. During both spring and neap tides, the highest and lowest H' was recorded at Station 2 (2.457 and 2.431) and Station 1 (1.858 and 1.992), respectively. On the other hand, both spring and neap tides showed the highest and lowest E at Station 2 (0.703 and 0.722) and Station 1 (0.518 and 0.619), respectively.

Table 4. Relative abundance of phytoplankton species at Stations 1, 2 and 3 during spring and neap tides.

	Spring tides			Neap tides		
	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3
Bacillariophyta						
Achnanthes spp.	+	-	-	-	-	-
Amphora spp.	-	-	+	-	+	-
Bacteriastrum spp.	-	-	+	-	-	-
Biddulphia spp.	+	-	++	+	-	-
Chaetoceros spp.	+	++	++	+	+++	+++
Climacodium spp.	-	-	+	-	-	-
Cocconeis spp.	+	+	-	-	-	+
Coscinodiscus spp.	++	++	+++	++	++	++
Cyclotella spp.	+++	+++	+++	+++	++	+++
Cylindrotheca spp.	++	+++	++	++	+++	+++
Cymbella spp.	+	+	+	-	+	-
Dinobryon spp.	-	-	-	+	-	-
Fragilaria spp.	+	+	+	-	+	+
Gomphonema spp.	+	-	-	-	-	+
Guinardia spp.	-	-	+	-	-	-
Gyrosigma spp.	++	++	++	+	++	++
Heterothrix spp.	+	+	+	-	-	-
Mallomonas spp.	-	-	-	+	-	-
Melosira spp.	-	+	+	+	++	+
Navicula spp.	++	++	++	++	++	++
Neidium spp.	-	+	-	+	+	+
Nitzschia spp.	++	+++	++	++	++	++
Pinnularia spp.	+	+	+	+	+	-
Pleurosigma spp.	-	+	+	-	+	-
Pseudo-nitzschia spp.	+	+	-	-	-	-
Rhizosolenia spp.	+	-	+	-	-	-
Skeletonema spp.	++	+	+	+	++	++
Surirella spp.	+	+	+	-	+	+
Synedra spp.	++	+	+	-	+	-
Synura spp.	+	-	+	+	-	-
Thalassionema spp.	+	-	+	-	-	-
Triceratium spp.	+	+	+	-	-	-
Dinophyta						
Alexandrium spp.	-	+	-	-	-	-
Ceratium spp.	-	-	+	-	-	-
Dinophysis spp.	++	++	+++	-	++	++
Gambierdiscus spp.	-	-	-	-	-	+
Gonyaulax spp.	-	+	++	-	++	++
Lingulodinium spp.	-	-	-	-	+	-
Pentapharsodinium spp.	-	-	+	-	-	-
Protoceratium spp.	-	+	+	-	++	++
Protoperidinium spp.	+	++	+	-	+	+

Contd...

Table 4. Continued.

	Spring tides			Neap tides		
	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3
Chlorophyta						
Actinastrum spp.	+	-	-	-	-	++
Chlorella spp.	+	+	-	++	+	++
Oocystis spp.	+	-	-	++	-	-
Ophiocytium spp.	-	-	+	-	-	-
Pediastrum spp.	+	-	-	+	-	-
Scenedesmus spp.	+	+	-	++	-	+
Euglenophyta						
Euglena spp.	+	+	+	++	-	-
Lepocinclis spp.	+	+	-	-	-	-
Phacus spp.	+	-	-	++	+	++
Trachelomonas spp.	++	+	+	+	+	+
Cyanophyta						
Anabaena spp.	-	-	-	++	-	+
Noctoc spp.	-	-	+	-	-	-
Oscillatoria spp.	++	+++	++	++	+++	++
Spirulina spp.	+	+	+	-	+	+
Cryptophyta						
Cryptomonas spp.	+++	++	+	+++	++	+++

(-): Absent; (+): Rare (0.01-1.00 %); (++): Average (1.01-10.00 %); (+++): Abundant (10.01-100.00 %)

Table 5. Dominant phytoplankton at Stations 1, 2 and 3 with their ISI values.

Species	ISI		
	Station 1	Station 2	Station 3
Chaetoceros spp.	0.01	1.30	1.99
Coscinodiscus spp.	0.74	0.74	2.93
Cryptomonas spp.	6.72	0.50	0.19
Cyclotella spp.	27.54	5.25	12.47
Cylindrotheca spp.	4.05	18.21	8.52
Gyrosigma spp.	0.27	1.39	0.31
Navicula spp.	2.55	1.45	0.66
Nitzschia spp.	0.98	6.39	0.53
Oscillatoria spp.	28.82	4.08	0.13

Table 6. Richness, Shannon-Wiener's diversity and evenness indices of phytoplankton at Stations 1, 2 and 3 during spring and neap tides.

Stations	Species Richness		Shannon Wiener's		Evenness	
	Spring	Neap	Spring	Neap	Spring	Neap
Station 1	36	25	1.858	1.992	0.518	0.619
Station 2	33	29	2.457	2.431	0.703	0.722
Station 3	37	27	2.242	2.283	0.621	0.693

Correlation between phytoplankton community and water quality parameters

Pearson's Correlation between dominant phytoplankton with water quality parameters are presented in Table 7. *Chaetoceros* spp. showed significant positive correlations with pH (r = 0.499, P < 0.00), dissolved oxygen (r = 0.428, P < 0.009), transparency (r = 0.345, P < 0.039) and nitrite (r = 0.344, P < 0.040). *Coscinodiscus* spp. had significant positive correlations with salinity (r = 0.625, P < 0.000), conductivity (r = 0.545, P < 0.001) and TSS (r = 0.514, P < 0.001). *Cryptomonas* spp. revealed significant negative correlations with salinity (r = 0.419, P < 0.011), conductivity (r = 0.456, P < 0.005) but showed significant positive correlations with light intensity (r = 0.352, P < 0.035). *Cyclotella* spp. had significant negative correlation with pH (r = 0.451, P < 0.006) and orthophospahte (r = 0.440, P < 0.007) but showed significant positive correlations with nitrate (r = 0.338, P < 0.044) and ammonia (r = 0.343, P < 0.041). *Cylindrotheca* spp. revealed significant positive correlations with transparency (r = 0.347, P < 0.038) and orthophosphate (r = 0.341, P < 0.042). *Gyrosigma* spp. showed significant positive correlations with conductivity (r = 0.362, P < 0.030) while *Nitzschia* spp. showed significant negative correlations with temperature (r = 0.383, P < 0.021).

Discussion

Tidal changes influenced various water parameters, thus affecting the composition and distribution of phytoplankton. Salinity was one of the most important water parameters that often functions as an ecological barrier and reliable indicator in an estuary system (Sodré *et al.* 2011). Higher salinity was observed during spring tides (Table 2 and Fig. 2d) with higher value at Station 3 and decreased towards Station 1 (Table 3) because Station 3 is situated at the mouth of the estuary which is closer to the sea. Station 3 received stronger flow of landward seawater resulting higher turbulent mixing which resulted in higher salt intrusion similarly reported by Hsu *et al.* (1999). Higher salt intrusion increased the conductivity similarly observed here (Table 3) possibly due to higher dissolved ions particularly sodium, chloride, magnesium, sulfate and calcium (Thomas 1986). Moreover, Station 1 was located in the upstream of the estuary and received fresh water influx from the river, thus resulting in lower salinity and conductivity (Table 3) as also observed by other studies (Prasanna & Ranjan 2010; Yap *et al.* 2011).

Greater changes of water level and turbulent mixing during spring tides also bring the nutrient to the water surface, affecting the phytoplankton distribution (Kunneke & Palik 1984). This is consistent with our study where higher nitrate and orthophosphate were detected during spring tides as can be seen in Table 2. In this study, the light intensity was similar (Table 2) throughout the sampling duration representing a typical phenomenon of tropical climates and do not seem to affect the phytoplankton composition and distribution as also reported by Magalhães *et al.* (2006) and Sodré *et al.* (2011). Higher surface nutrients observed in this study during spring tides probably stimulated higher photosynthetic activity, hence resulted in higher abundance of phytoplankton (except for Station 2) (Fig. 4) and richness (Table 7). Higher nutrients during spring tides could have been caused by the inundated mangrove forest and river runoffs similarly reported by Tanaka & Choo (2000). This corresponded to higher suspended solids and lower transparency and dissolved oxygen in this study (Table 2) and in similar studies (Chen *et al.* 2006; Moskalski & Torres 2012). Higher suspended solids with lower transparency could have limited the light penetration and phytoplankton production despite the high nutrient content. However, high abundance of phytoplankton observed in this study may represent the dominant species competitiveness in light limiting condition corresponded to lower diversity (Table 7), similarly reported by Canini *et al.* (2013) in tropical mangrove estuary of Philippines. Interlandi & Kilham (2001) suggested that light limiting factor in eutrophic systems only favours the growth of certain species resulting in higher abundance and lower diversity.

Lower chlorophyll-*a* was observed during spring tides compared to neap tides in this study (Table 2). This could be related to the dilution of chlorophyll-*a* from the increased inflow of seawater into the estuary during spring tides compared to neap tides. On the other hand, higher chlorophyll was observed during neap tides in Serangoon Harbor of Singapore (Ooi *et al.* 2010), probably the result of reduced water mixing and tidal flushing where the water is calmer. Reduced water mixing and tidal flushing helps the precipitation of nutrients, hence encouraged algal bloom resulting in higher chlorophyll concentration.

Table 7. Pearson's correlation coefficients between water quality parameters and density of some frequently occurring phytoplankton genus.

Species	Temp	pH	DO	Salinity	Conductivity	Light intensity	Transparency	TSS	Chl *a*	NO_2	NO_3	NH_3	PO_4
Chaetoceros spp.	-0.077	0.499**	0.428**	0.193	0.208	-0.193	0.345*	0.045	0.050	0.344*	-0.299	-0.265	0.002
Coscinodiscus spp.	0.038	0.050	-0.191	0.625**	0.545**	-0.116	-0.155	0.514**	-0.239	0.049	-0.322	-0.321	0.215
Cryptomonas spp.	0.245	-0.072	-0.009	-0.419*	-0.456**	0.352*	-0.237	-0.302	0.242	-0.171	0.309	0.268	-0.290
Cyclotella spp.	0.046	-0.451**	-0.263	-0.172	-0.164	-0.003	-0.210	-0.278	-0.001	-0.004	0.338*	0.343*	-0.440**
Cylindrotheca spp.	0.146	0.177	0.095	0.120	0.236	0.037	0.347*	0.022	0.209	0.213	-0.296	-0.275	0.341*
Gyrosigma spp.	0.263	-0.028	-0.032	0.199	0.362*	-0.076	-0.042	0.324	-0.008	-0.128	-0.262	-0.211	0.326
Navicula spp.	0.216	-0.157	-0.156	-0.134	-0.093	-0.122	-0.223	-0.185	-0.184	0.186	0.229	0.160	-0.303
Nitzschia spp.	-0.383*	0.095	-0.007	0.166	0.268	0.003	0.065	0.286	-0.082	0.049	-0.148	-0.152	0.186
Oscillatoria spp.	0.291	0.290	-0.042	0.158	0.258	0.078	0.243	0.070	-0.089	0.183	-0.257	-0.229	0.323

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)
Temp: temperature; TSS: total suspended solids; Chl *a*: chlorophyll-*a*; NO_2: nitrite; NO_3: nitrate; NH_3: ammonia; PO_4: orphophosphate.

Changes of water quality during spring-neap tides influenced the composition and distribution of phytoplankton in this study. Dinophyta, composed of marine dinoflagellates, were dominant in number in the downstream (Station 3) nearer to the sea with higher salinity followed by Stations 2 and 1 (Table 4). This was because Dinophyta are known to prefer calm water (Waite 1996) and the freshwater discharge in Merbok river estuary was stronger during neap tides due to reduced landward seawater flow (Ong *et al.*1991). On the other hand, Chlorophyta and Euglenophyta being dominant freshwater species were found mostly in the upstream (Station 1) followed by Stations 2 and 3 in the present study (Table 4). Bacillariophyta, Cyanophyta and Cryptophyta have high tolerance towards salinity variation and can be found in similar quantity in both freshwater and marine environment (Table 4). The inflow of water into the estuary during spring tides could have resulted in higher diversity of phytoplankton in this study similar to the Amazon estuaries as reported by Sodré *et al.* (2011). Spring tides recorded higher abundance of phytoplankton (Fig. 4) and richness (Table 7) for both fresh water and seawater species. Six groups of phytoplankton were identified along Merbok river estuary according to dominance: Bacillariophyta, Dinophyta, Chlorophyta, Euglenophyta, Cyanophyta and Cryptophyta with a total of 56 genera. Dominance of Bacillariophyceae contributed about 20 to 25 % of the world net primary production (Werner 1977). Marshall *et al.* (2005) also reported dominance of Bacillariophyceae with 1454 species of phytoplankton in Chesapeake Bay and tidal regions of its major tributaries. The most dominant genera at Stations 1, 2 and 3 were *Oscillatoria* spp., *Cylindrotheca* spp. and *Cyclotella* spp., respectively, while abundance of *Cyclotella* spp. and *Cylindrotheca* spp. were high at all stations. Most genera observed in Merbok river estuary were diatoms similarly observed elsewhere (Ganjian *et al.* 2010; Sodré *et al.* 2011). Diatom species such as *Cyclotella* sp. was one of the important bioindicator of aquatic pollution (Shruthi *et al.* 2011) and has been used to monitor pollutions in streams (Wan Maznah & Mansor 2002). In addition, *Cylindrotheca* sp. and *Nitzschia* sp. were bioindicators in wastewater from human activities and described as opportunists resistant to constant changes in concentrations of nutrient (Álvarez-Góngora & Herrera-Silveira 2006). *Cyclotella* spp., *Cylindrotheca* spp. and *Nitzschia* spp. found in Merbok river estuary (Table 5) were among the important genera listed in the ISI in this study (Table 6) commonly used as water quality bioindicator (Álvarez-Góngora & Herrera-Silveira 2006; Shruthi *et al.* 2011; Wan Maznah & Mansor 2002). This could mean that the environmental condition in Merbok river estuary is deteriorating which could have been caused by the nutrients flow and runoffs from nearby aquaculture ponds and cages, oil plantations, villages as well as small industries and developments that need continuous monitoring.

The trends of phytoplankton composition and distribution in Merbok river estuary were influenced by differences in locality and velocity of water movement. During spring tides, higher abundance of phytoplankton was found at Station 1 (upstream) followed by Station 2 (midstream) and Station 3 (downstream) (Fig. 4). O'Boyle & Silke (2010) stated that tidal range, river flow and morphological characteristics of the water body determined the level of mixing between freshwater and seawater. According to Ong *et al.* (1991), Merbok river estuary is a partially mixed coastal plain estuary and the tidal flux is greater than the freshwater discharge. Therefore, it is possible that the landward seawater prevents the seaward freshwater discharging from the upstream and the landward seawater has sufficient tidal force to push the phytoplankton upstream. Moreover, there are 12 tributaries (Baharu river, Bujang river, Batu river, Semeling river, Bangkok river, Lalang river, Tukang river, Petani river, Pasir river, Teluk Wang Besar river, Kerisik river and Keluang river) and ground runoff which might contribute to the freshwater input of nutrients during heavy rainfall into the upstream of Merbok river (Kamrudzaman *et al.* 2012; Ong *et al.* 1991). As a result, restriction of freshwater discharge from the system and contribution of freshwater input from the tributaries might lead to the accumulation of phytoplankton in the upstream.

In this study, considerably high abundance of phytoplankton was found at Station 1 (Fig. 4) correlated with high ammonium and nitrate but low nitrite concentration (Table 3). This was probably due to higher preferences towards ammonia for phytoplankton growth. As the ammonia concentration becomes low, nitrate will be utilized instead but at the same time nitrite were converted to nitrate through nitrification. When the phytoplankton dies, ammonia will be the end product of decomposition and the cycle continues (Ooi *et al.* 2010; Rajasegar 1998). On the other hand, phosphorus, one of the necessary nutrients for phytoplankton growth, was low at Station 1 compared to Stations 2 and 3 in this study. It might be related to higher phosphorus absorption in the form of soluble inorganic orthophosphate at Station 1 with high abundance of phytoplankton. In addition, point sources of inorganic orthophosphate which include fertilizers, pesticides and detergents could have been contributed from nearby farms, plantation and villagers municipal and industrial wastewater treatment facilities.

As a result of higher nutrient content in the upstream (Station 1), higher abundance of phytoplankton observed here coincided with lower transparency, dissolved oxygen and pH. This could probably be related to eutrophication (Nagy *et al.* 2002) where high ammonia and nitrate encourages phytoplankton and algal growth to a point that they absorb dissolved oxygen during respiration (Yap *et al.* 2011) and release carbon dioxide that decreased the pH (Eddy 2005) as well as reducing light penetration into the water. Death of other aquatic plants and animals probably due to eutrophication and lack of oxygen as well as decomposition of organic matter further depleted the oxygen levels and lowered the pH. Similar phenomenon was reported by Alongi *et al.* (2003) where fast bacterioplankton growth rates showed rapid decline of oxygen level and pH especially at night. Despite the trends in water quality parameters affected by tidal changes on the phytoplankton composition and distribution, dissolved oxygen, water temperature, TSS and orthophosphate are still with in the range of Class E of Marine Water Quality Criteria and Standard suggested by the Department of Environment Malaysia (DOE 2011). Class E is a standardized water quality category aimed to monitor the environmental condition of mangroves, estuaries and river-mouth waters in Malaysia. Different types of water bodies are categorized into different classes according to its beneficial uses. Class 1 is categorized for preservation, marine protected areas and marine parks, Class 2 focused on the marine life, fisheries, coral reefs, recreational and mariculture activities while Class 3 refers to the environmental condition set for ports, oil and gas fields. The appropriate set of guidelines for safe and potable water quality in Malaysia was published in 1983 following the expert guidance from the World Health Organization, Western Pasific Regional Centre for the Promotion of Environmental Planning and Applied Studies (WHO)/PEPAS. The level of water qualities in this study is still acceptable as specified in Class E standards as follows: dissolved oxygen (4.0 mg L^{-1}), water temperature ($\leq$ 2 °C increase over maximum ambient), total suspended solids (100 mg L^{-1}) and orthophosphate (0.79 µM). However, nitrite, nitrate and ammonia contents were higher in this study compared to the standards, 1.20, 0.97 and 4.11 µM, respectively.

Different water quality parameters may favour different types of phytoplankton as a result of having different favourable ranges of environmental requirement for growth (Fahnenstiel *et al.* 1995; Litchendorf 2006). Positive correlations of *Chaetoceros* spp. with pH reported here were similar to the results reported by Thornton (2009), who stated that photosynthetic efficiency of *Chaetoceros* spp. decreased with increased acidity. Bunt (1971) revealed the importance of dissolved oxygen in photosynthetic carbon fixation where *Chaetoceros* spp. were inhibited at low carbon dioxide with the lowest dissolved oxygen levels, coincides with positive correlations of dissolved oxygen in this study. Kuwata *et al.* (1993) reported that both dormant and photosynthesizing *Chaetoceros* spp. possessed lower amount of chloroplast in its cell and requires sufficient light to germinate. This is similarly reported here where *Chaetoceros* spp. were positively correlated with transparency possibly for better light penetration. *Chaetoceros* spp. also showed higher correlations with nitrite in this study coincides with higher abundance at Station 3 containing higher nitrite content. Being a marine genus, *Coscinodiscus* spp. showed positive correlations with salinity and conductivity, in contrast with *Cryptomonas* spp., a fresh water species. Since *Cryptomonas* spp. grow better in deeper water, they require higher light intensity for better penetration, as shown by the positive correlations with light intensity observed in this study. *Cyclotella* spp. are diatoms which can be found in high nutrient environment (Yang *et al.* 2005), similarly reported here with positive correlations with nitrate and ammonia content but negatively correlated with orthophosphate.

Conclusions

Spring and neap tidal changes were observed to influence the water quality in Merbok river estuary at different location (upstream, midstream and downstream), which in turn affected the composition and distribution of phytoplankton. Although the presence of certain important diatoms used as a bioindicator for aquatic pollution was detected in this study, the status of most water quality parameters are still within the Malaysian standard range. In short, water quality varied according to the tidal events, which subsequently affected the composition and distribution of phytoplankton, thus reflecting the importance of biotic and abiotic parameters in understanding the overall health status of Merbok river estuary.

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