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Effects of Environmental Conditions and Nutrients on the Occurrence and Distribution of Potentially Harmful Phytoplankton in Mesotrophic Water

(Kesan Keadaan Alam Sekitar dan Nutrien ke atas Kewujudan dan Pengagihan Fitoplankton Berpotensi Berbahaya di dalam Air Mesotrofik)

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ABSTRACT

*Marudu Bay, north coast of Sabah is characterized with mesotrophic water body and typical environmental parameters throughout the year. The current study was undertaken to evaluate the effect of environmental parameters and nutrients in mesotrophic water on the occurrence and distribution of potentially harmful phytoplankton species. The samplings were conducted over a period of thirteen months, covering southwest monsoon (SWM), inter-monsoon (IM), and northeast monsoon (NEM), at ten stations throughout the bay. Physical parameters (temperature, salinity, pH, dissolved oxygen, current speed and secchi depth), biological parameters (cell densities of phytoplankton) and chemical parameters (phosphate, nitrate, silicate and ammonia) were examined. The results indicated at least eight potentially harmful phytoplankton species (*Dinophysis caudata, D. miles, Ceratium furca, C. fursus, Prorocentrum micans, P. sigmoides, P. triestinum *and* Pseudo-nitzschia *sp.) were detected in north coast of Sabah. However, the potentially harmful phytoplankton species contributed only about 1.3% of the total phytoplankton community. Under nutrient deprivation conditions, the potentially harmful phytoplankton species distribution was mainly influenced by the ability to utilize other nitrogen sources, cell mobility and toleration to low nutrients environments.*

Keywords: Environmental parameters; nutrients; potentially harmful phytoplankton species; species composition

ABSTRAK

*Teluk Marudu, pantai utara Sabah dicirikan dengan air mesotrofik dan parameter alam sekitar khas sepanjang tahun. Kajian ini telah dijalankan untuk menilai kesan parameter alam sekitar dan nutrien dalam air mesotrofik dan taburan spesies fitoplankton yang berbahaya. Sampel telah dijalankan dalam tempoh tiga belas bulan, yang meliputi monsun barat daya (SWM), peralihan monsun (IM) dan monsun timur laut (MEB) pada sepuluh stesen di seluruh teluk. Parameter fizikal (suhu, kemasinan, pH, oksigen terlarut, kelajuan semasa dan kedalaman secchi), parameter biologi (kepadatan sel fitoplankton) dan parameter kimia (fosfat, nitrat, silikat dan ammonia) telah diperiksa. Hasil kajian telah menunjukkan sekurang-kurangnya lapan spesies fitoplankton yang mungkin berbahaya (*Dinophysis caudata, D. miles, Ceratium furca, C. fursus, Prorocentrum micans, P. sigmoides, P. triestinum *dan* Pseudo-nitzschia *sp.) dikesan di pantai utara Sabah. Walau bagaimanapun, spesies fitoplankton yang berbahaya hanya menyumbang kira-kira 1.3% daripada jumlah komuniti fitoplankton. Dalam keadaan kekurangan nutrien, pengedaran spesies fitoplankton berbahaya terutamanya dipengaruhi oleh keupayaan untuk menggunakan sumber nitrogen lain, pergerakan sel dan toleransi nutrien persekitaran rendah.*

Kata kunci: Komposisi spesies; nutrien; parameter alam sekitar; spesies fitoplankton berbahaya

INTRODUCTION

Over the last several decades, a global escalation of harmful algal blooms (HABs) has been reported in the marine coastal environments (Cordier et al. 2000). In coastal waters of Sabah, two HAB species, *Pyrodinium bahamense* var. *compressum* and *Cochlodinium polykrikoides* have frequently been reported to affect the economy of the state and brought fear to local community (Mohammad-Noor et al. 2012). The first harmful algal blooms event in Malaysia was reported in 1976. A marine dinoflagellate called *P. bahamense* var. *compressum* was bloomed in Brunei Bay on the west coast of Sabah (Roy 1977). Several people were poisoned after consuming shellfish from the related areas. The blooms of this species have continued to occur almost annually and spread to other parts of Sabah water mainly in Sipatang, Kuala Penyu, Binsuluk and Kota Kinabalu (Anon 2001). The first evidence of *C. polykrikoides* was in 2005 and the occurrence of this species has been associated with coastal waters red discolorations and fish mortality in aquaculture (Anton et al. 2008). To date, the HAB problems have only been reported along the coastal water of the west coast of water. Therefore, HABs research in Sabah has been carried out and focus in west coast of Sabah. However, the occurrence of *P. bahamense* var. *compressum* in the east coast of Sabah has been reported in 1998 (Anton & Suibol 1999). Later, another study has identified eight more potential HAB species at the east coast of Sabah which suggest possibility of HAB problem (Mohammad-Noor et al. 2012). Whereas for the north coast of Sabah, very limited information on the occurrence and distribution of HAB or potential HAB species are available. The terms 'potential HAB species' used in current study refer to HAB species that reported harmful in other geographical regions but no record in Malaysia so far.

Eutrophication and a shift in macronutrients (nitrogen: phosphorus; N:P) ratios by anthropogenic sources have been suggested to be the main causative factor of increasing HABs events (Anderson et al. 2002; Smayda 1990). Many studies also concluded the positive relation between coastal nutrient over-enrichment and the increase of HABs events (Anderson et al. 2002; Anton et al. 2008; Hallegraff 1993). In the eutrophic water, the N:P ratio is usually lower (around 10:1 or lower) than the Redfield ratio (16:1) (Redfield et al. 1963; Smith 2006). Nevertheless, HABs cell density has been shown to negatively correlate with N: P ratios (Hodgkiss & Ho 1997). For example, decreasing N:P ratio in Tolo Harbor from 20:1 to 11:1 has shown to increase the abundance of HAB species whereby the optimal N:P ratios for *Prorocentrum micans*, *P. sigmoides* and *P. triestinum* were recorded at 5-10, 4-15 and 8-15 (Hodgkiss 2001). Similarly, the nutrient supply does not necessarily correlate with the rate of nutrient assimilation by phytoplankton, because the nutrient uptake capability of organisms are vary (Anderson et al. 2002). Environmental parameters also significantly influence the occurrence (Anathan et al. 2008) and composition (Agboola et al. 2011) of phytoplankton and HABs. This is because the effectiveness of nutrient assimilation by phytoplankton depends on several environmental factors such as light, temperature, salinity, and water column stability (Anderson et al. 2002; Tan & Ransangan et al. 2015). Growth of *Pyrodium bahamense* var. *compressum* (Anton et al. 2000) and *A. tamarense* (Lim & Ogata 2005) were positively associated with water salinity and temperature, respectively. In addition, blooms of *Karenia* sp. was also believed to be influenced by water temperature (Dahl & Tangen 1993), while in Tunisian lagoon, the bloom of a *Gynodinium aureolum* was associated with decreasing day length (Romdhane et al. 1998).

Under oligotrophic or mesotrophic conditions, HAB cells in aquatic environments exist at a negligible concentration and therefore are usually harmless (Tan & Ransangan 2015). In eutrophic water, certain conditions such as environmental factors or nutrient ratios have been known to trigger HABs to bloom or secrete a high concentration of potent toxins (Deeds et al. 2008). To date, very limited information is available on the effects of environmental conditions and nutrients in mesotrophic and oligotrophic waters on the abundance and composition of HABs species. Therefore, the current study was carried out to determine the occurrence of HAB and potential HAB species in the north coast of Sabah and evaluate the relationship between environmental parameters and distribution of HABs in a mesotrophic environment.

Materials and Methods

Study Area

Marudu bay is situated on the north of Sabah, 6° 35' to 7° N and longitude of 116° 45' to 117° E (Figure 1). It has an equatorial climate with uniform temperature, high humidity and copious rainfall due to its proximity to the equator (Malaysian Meteorological Department 2014). Ten sampling stations were located based on water depth and anthropogenic influences. Stations 1, 2, 3 and 10 were located at the areas with water depth not more than 5 m, stations 4, 5, 7, 8 and 9 at water depth in the range of 5 to 10 m and station 6 is located at water depth of 21 m. For influences of anthropogenic factors, stations 1, 2 and 10 were adjacent to high human density area, where station 1 is the nearby the green mussel farm. Station 3 was located in front of a river mouth and also the main artisanal fishing ground, while many anchovy fishing platforms were found around the stations 7 and 8.

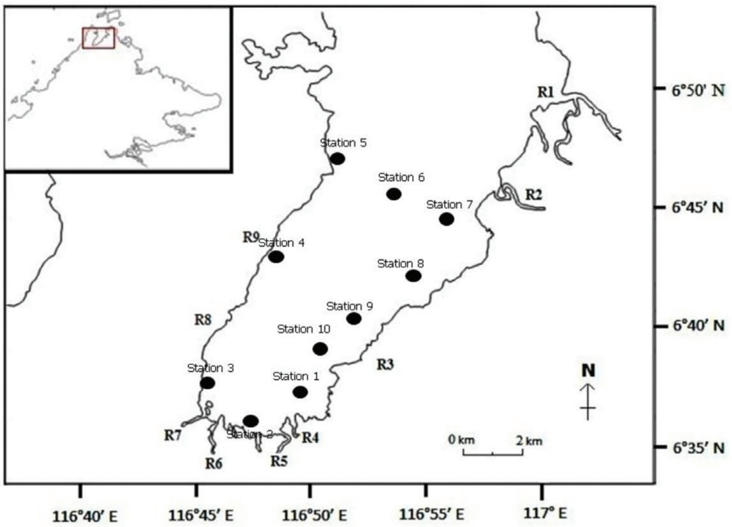
Sampling Schedule

Sampling were conducted once monthly from April 2014 until April 2015 (thirteen months). The sampling periods covered the Southwest monsoon (SWM) (May to September), Inter-monsoon (IM) (April & October) and Northeast monsoon (NEM) (November to March). North coast of Sabah, the Marudu Bay usually experiences heavy rainfall during NEM, while limited rainfall during SWM (Malaysian Meteorology Department 2014).

Sample Collection

Qualitative samples of phytoplankton were collected by vertical tow of plankton net (mesh size 20 μm) to cover the depth range of 3 m. The net was towed several times from 3 m depth to the surface of the water until the water in the sample collector becomes unclear or coloured by the concentrated algae (about 200 ± 50 L, (Mean ± SD) of sea water in average has been filtered in each sample). The sample was then immediately preserved with Lugol's solution (Saraceni & Ruggiu 1974). Species identification was performed using a Carl Zeiss light microscope at 400× and 1000× magnification according to Hartley (1996).

At each station, 1 L seawater samples were collected at 0.5 m depth using a 1-L Van Don water sampler and immediately preserved with Lugol's solution (Saraceni & Ruggiu 1974) for phytoplankton quantification. In laboratory, samples were concentrated using Utermöhl sedimentation method (24 h) into 50 mL (Utermöhl 1958). The phytoplankton and HAB cell densities were counted using a Sedgwick Rafter chamber at 400× magnification. Environmental parameters at 0.5 m below the water surface, including temperature, salinity, pH and dissolved oxygen were measured using a multi-functional environmental sensor (Hydrolab; Loveland, CO, USA). Water current was measured using current meter (Stanley USA) and transparency as secchi disk depth was measured by secchi



Note: R1= Pitas River; R2= Bangkoka River; R3= Marasimsim River; R4= Taritipan River; R5= Raku River; R6= Sumbilingan River; R7= Marudu River; R8= Karangawan River; R9=Matunggong River

FIGURE 1. Location of the nine sampling stations in Marudu Bay

disc. Subsurface water samples of 1 L were collected and pre-filtered immediately through 0.45 µm pore-size GF/C membrane filters (Whatman). The filtrates were stored at 4°C until analyzed. The nutrients, phosphate (PO_4-P), nitrate (NO_3-N), ammonia (NH_4-N) and silica (SiO) were measured following Parsons et al. (1984).

SECONDARY DATA

Secondary data of monthly amount of rainfall and wind speed were obtained from Meteorology Department, Kota Kinabalu.

STATISTICAL ANALYSIS

Statistical analyses were performed using the SPSS Windows Statistical Package (version 21). The tests were judged to be significant at $p<0.05$ level. Prior to analyses, all variables were tested for normality and homogeneity of variances. One-way ANOVA was performed to test for significant differences in environmental variables, nutrients and phytoplankton cell density among stations and months. Fourteen environmental variables were reduced into five common factors using principal factor method. To help the interpretation of the factors, varimax rotation was performed (Wang et al. 2008). Correlation coefficients were calculated between main environmental variables, factor scores and logarithmic transformation ($\log_{10}(n+1)$) of biological parameters (Total phytoplankton and potentially harmful phytoplankton species) (Aktan et al. 2005).

RESULTS

ENVIRONMENTAL PARAMETERS AND NUTRIENTS

The spatial and temporal variations of environmental parameters at Tanjung Batu water are illustrated in Figures 2 and 3, respectively. In general, no significant differences ($p>0.05$) were recorded in temperature (29.2 to 30.2°C), salinity (29.2 to 31.2 psu), pH (8.0 to 8.2), dissolved oxygen (5.3 to 5.6 mg/L) and current speed (22.2 to 31.4 m/min) throughout the stations. However, the visibility in the measurement of secchi depth in bay pocket, stations 1, 2, 3, 4 and 10 (1.3 to 2.5 m) was significantly lower (°<0.05) than that in other stations (4.2 to 10.2 m).

For temporal variations, significant differences were recorded in all environmental variables. Water temperature in May to December 2014 (29.5 to 31.2°C) was significantly higher ($p<0.05$) than that in January to March 2015 (28.0 to 28.2°C). Salinity in April to October 2014 (30.2 to 33.8 psu) was significantly higher ($p<0.05$) than that in November 2014 to February 2015 (24.3 to 29.6 psu). Dissolved oxygen in April to October 2014 (5.3 to 7.0 mg/L) was significantly higher ($p<0.05$) than that in November 2014 to January 2015 (3.6 to 3.8 mg/L). Current speed in April to September 2014 (32.6 to 44.5 m/min) was significantly higher ($p<0.05$) than that in October 2014 to March 2015 (8.6 to 17.3 m/min). pH value in May and June 2014 (8.3 to 8.4) was significantly higher ($p<0.05$) than that in other months (7.9 to 8.2). Water visibility in the measurement of Secchi depth in May to July 2014 (4.5 to 6.9 m) was significantly higher ($p<0.05$) than that in other months (1.3 to 4.0 m). Wind speed in January to March (2.8 to 3.1 m/s), July (2.8 m/s) and October 2014 (2.7 m/s) was significantly higher than that in other months (1.9 to 2.5 m/s).

The nutrients concentration is summarized in Table 1. The dissolved inorganic phosphorus (DIP), total dissolved inorganic nitrogen (DIN) and dissolved silica (DSi) concentrations ranged from 0 to 3.19, 0.23 to 35.91 and 0.29 to 20.79 µM, respectively. The nutrients ratio, N:P, Si:N and Si:P ratios ranged from 0.58 to 334.25,

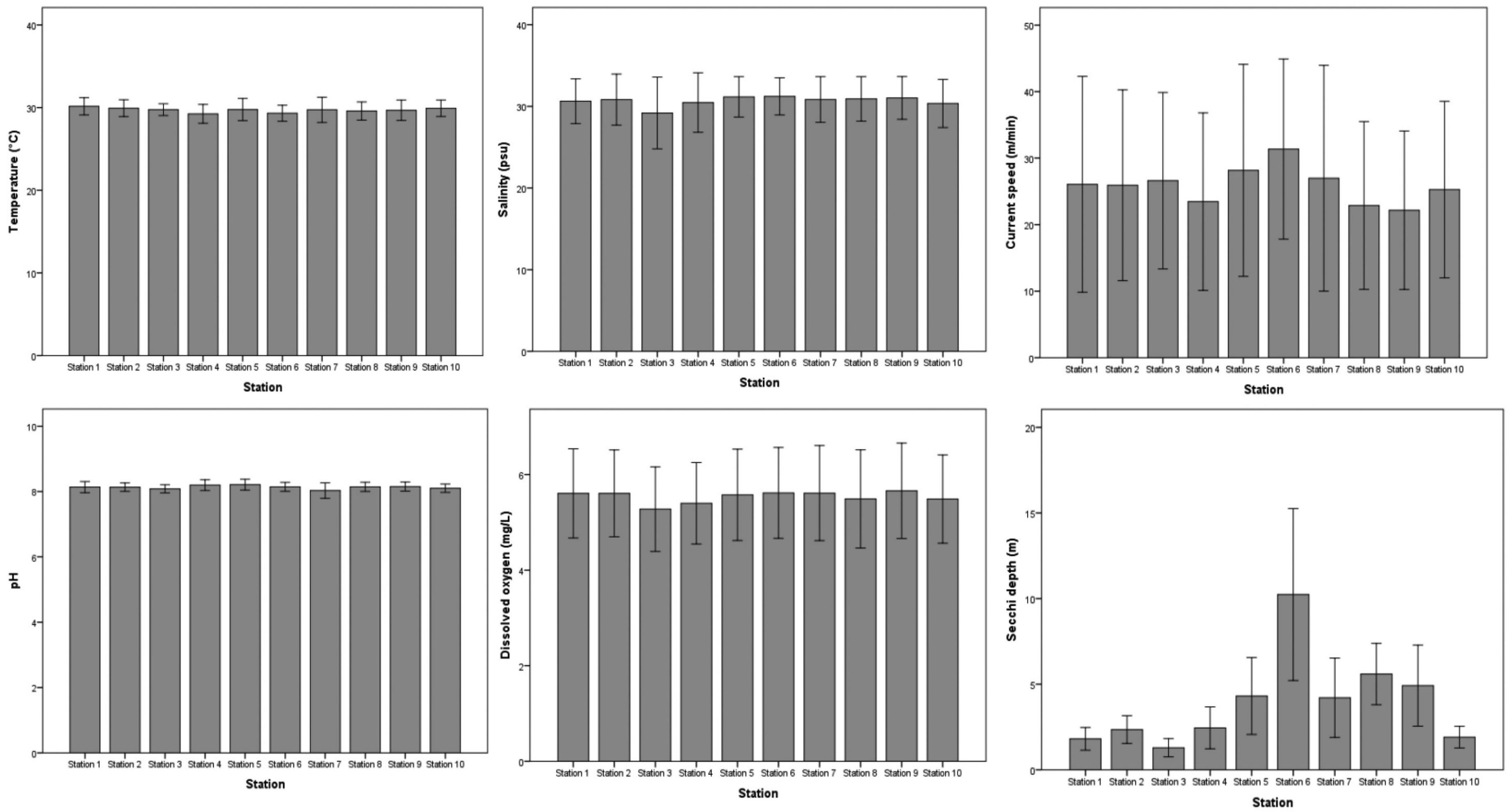


FIGURE 2. Spatial variations of environmental parameters in Marudu Bay recorded from February 2014 to January 2015

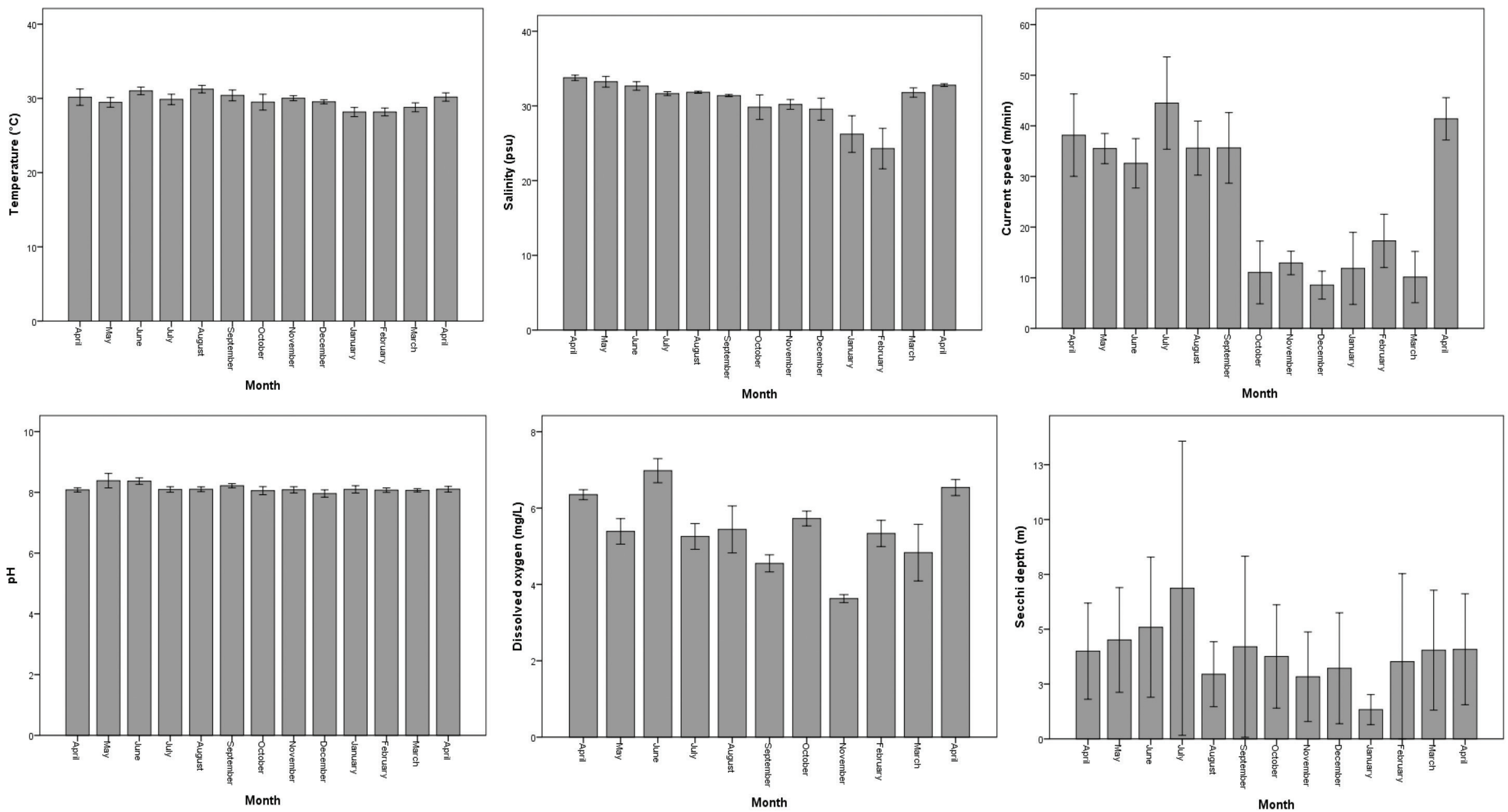


FIGURE 3. Temporal variations of environmental parameters in Marudu Bay recorded from February 2014 to January 2015

0.01 to 24.34 and 0.67 to 475.76, respectively. In general, no significant differences (p>0.05) were recorded in all nutrients concentration throughout the stations.

For temporal comparisons, nitrate in April to May 2014, November 2014 and January 2015 (0.38 to 0.77 μM) was significantly higher (p<0.05) than that in any other months (0.05 to 0.17 μM). Nitrite in January 2015 (0.08 μM) was significantly higher (p<0.05) than that in other months (0.01 to 0.04 μM). Ammonia was significantly higher (p<0.05) in April and May 2015 (4.8 to 5.5 μM) than that in October 2014 to March 2015 (0.26 to 1.73 μM). DIN was highest (p<0.05) in April to May 2014 (5.8 to 6.0 μM). In addition, the DIN concentration recorded in June to September 2014 (2.6 to 3.9 μM) was also significantly higher (p<0.05) than that in October 2014 to March 2015 (0.45 to 1.42 μM). Silica was significantly higher (p<0.05) in May 2014 (6.5 μM) and January to February 2015 (3.8 to 4.0 μM) than that in other months (0.7 to 2.7 μM). Phosphate concentration was significantly higher (p<0.05) in May to June 2014 (0.35 to 0.80 μM) than that in other months (0.03 to 0.17 μM).

Marudu Bay received heavy rain in October 2014, December 2014 (maximum: 502.1 mm) and January 2015, while lowest rainfall of 1.1 mm in March 2015 (Figure 4). Mean wind speed recorded in January to March 2015 (3 to 3.1 m/s) was significantly higher than that the wind speed recorded in April to June, August 2014 and November 2014 (1.9 to 2.2 m/s).

PHYTOPLANKTON AND POTENTIALLY HARMFUL PHYTOPLANKTON SPECIES

The total phytoplankton cells density in Marudu Bay ranged between 5.1 and 194.8 × 10^3 cells/L. The phytoplankton community in Marudu Bay was dominated (69.8%) by Chaetocerotaceae (Table 2). Phytoplankton cell densities in stations 1, 2 and 10 (53.3 to 69.7 × 10^3 cells/L) was significantly higher (p<0.05) than that in other stations (13.0 to 38.8 × 10^3 cells/L). For temporal variations (Table 3), phytoplankton cell densities was highest (p<0.05) in September 2014 (196.6 × 10^3 cells/L). Moreover, the phytoplankton cell density in June (55.1 cells/mL) was also significantly higher (p<0.05) than that in August 2014, November 2014 and January to May 2015 (2.8 to 20.8 × 10^3 cells/L).

Total of eight potentially harmful phytoplankton species were identified in Marudu Bay. These included *Dinophysis caudata, D. miles*, *Ceratium furca*, *C. fursus*, *Prorocentrum micans*, *P. sigmoides, P. triestinum* and *Pseudo-nitzschia* sp. The abundance of potentially harmful phytoplankton species was about 1.3% of the total phytoplankton community, where *P. micans, C. furca, Psedonitzschia* sp. and *D. caudata* accounted for 27.7, 26.8, 25.9 and 5.4% of the total potentially harmful phytoplankton community. No significant difference (p<0.05) was recorded in cell density of potentially harmful phytoplankton throughout the stations. However for temporal comparisons, the cell density of potentially

TABLE 1. Temporal and spatial variations of mean nutrients concentration and its ratio recorded in Marudu Bay from February 2014 to January 2015

	Nutrients	NO2-N (μm)	NO3-N (μm)	NH4-N (μm)	DIN (μm)	DIP (μm)	DSi (μm)	N/P	Si/N	Si/P
	Station 1	0.036±0.036	0.030±0.040	3.18±6.11	3.51±6.14	0.14±0.14	3.22±2.92	24.67±19.14	3.63±5.17	54.34±54.84
	Station 2	0.050±0.050	0.034±0.038	2.17±2.09	2.56±2.11	0.12±0.13	2.88±2.44	30.41±24.64	2.41±2.82	62.40±107.75
	Station 3	0.046±0.040	0.026±0.031	2.12±1.99	2.42±2.09	0.18±0.26	2.81±1.40	22.63±18.90	2.45±2.36	38.03±39.50
	Station 4	0.025±0.025	0.025±0.026	2.29±2.09	2.56±2.14	0.15±0.23	2.23±1.49	29.89±20.37	1.72±1.62	43.43±45.68
	Station 5	0.021±0.013	0.13±0.11	1.84±1.45	1.98±1.48	0.14±0.16	2.15±1.33	22.13±17.55	1.88±1.73	31.92±25.45
	Station 6	0.023±0.016	0.16±0.15	1.73±1.56	1.95±1.61	0.13±0.16	1.94±1.09	23.90±14.29	1.71±1.53	34.55±30.96
	Station 7	0.025±0.017	0.14±0.14	1.97±2.02	2.13±1.96	0.12±0.13	1.97±1.26	23.94±22.00	2.01±2.36	30.69±26.78
	Station 8	0.031±0.018	0.18±0.20	1.76±1.69	1.98±1.80	0.14±0.20	1.96±1.30	20.79±15.44	2.10±2.43	29.78±25.07
	Station 9	0.021±0.012	0.38±0.71	2.23±2.33	2.63±2.65	0.30±0.70	2.61±3.16	29.32±28.82	1.89±2.16	31.06±27.40
	Station 10	0.031±0.024	0.21±0.22	1.90±1.91	2.14±2.01	0.19±0.27	3.64±3.20	39.46±75.93	3.00±2.59	56.74±69.00
IM	Apr	0.041±0.038	0.77±0.70	4.85±1.33	5.84±1.06	0.14±0.09	2.47±1.16	73.48±78.32	0.43±0.20	41.83±70.09
	May	0.030±0.015	0.48±0.45	5.53±0.80	6.04±0.93	0.36±0.17	6.54±4.36	22.31±14.03	1.08±0.69	23.21±20.21
SWM	June	0.022±0.021	0.05±0.03	2.49±0.76	2.56±0.77	0.80±0.65	2.09±0.80	4.81±3.08	0.94±0.60	3.58±2.05
	July	0.012±0.012	0.11±0.07	0.52±0.26	2.64±0.30	0.17±0.05	1.85±0.43	16.84±5.51	0.70±0.12	11.76±4.44
	Aug	0.030±0.020	0.09±0.04	2.88±0.34	3.00±0.33	0.11±0.04	0.73±0.25	31.64±10.93	0.25±0.09	7.50±3.09
	Sept	0.034±0.020	0.09±0.04	3.81±6.74	3.93±6.75	0.11±0.12	1.40±1.06	36.97±26.14	0.81±0.65	21.36±15.36
IM	Oct	0.026±0.013	0.17±0.18	0.26±0.07	0.45±0.21	0.05±0.04	2.73±2.68	13.89±12.10	6.17±5.42	89.90±121.62
	Nov	0.023±0.012	0.38±0.11	0.27±0.05	0.67±0.11	0.06±0.02	1.14±0.56	13.92±6.71	1.72±0.88	23.14±14.39
	Dec	0.033±0.016	0.17±0.10	0.34±0.08	0.54±0.13	0.05±0.03	2.03±1.03	14.90±9.78	3.70±1.46	49.51±34.08
NEM	Jan	0.081±0.057	0.48±0.32	0.26±0.06	0.82±0.35	0.06±0.02	4.02±0.70	13.63±6.56	5.76±2.36	69.93±25.20
	Feb	0.023±0.014	0.09±0.05	0.74±0.16	0.85±1.56	0.04±0.01	3.82±0.87	23.58±10.41	4.62±1.27	104.98±45.74
IM	Mar	0.012±0.007	0.12±0.04	1.28±0.17	1.42±0.18	0.03±0.02	1.37±0.29	53.58±20.03	0.99±0.24	54.58±25.93
	Apr	0.034±0.036	0.06±0.04	1.74±2.15	1.83±2.13	0.08±0.03	2.31±0.60	22.64±24.60	2.10±1.13	30.10±9.04

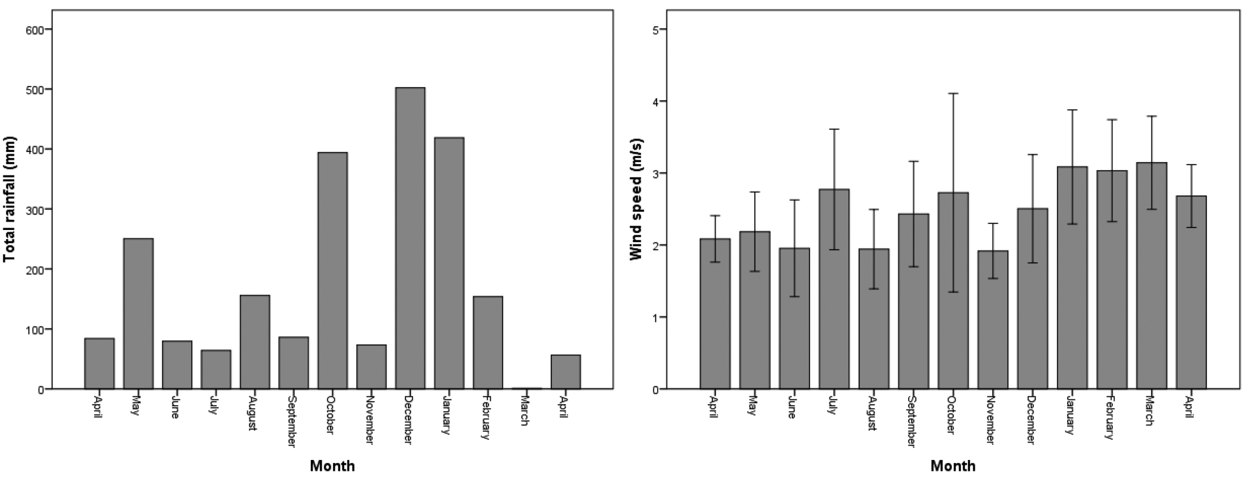


FIGURE 4. (A) total rainfall and (B) mean wind speed (mean± SD) in Marudu Bay from April 2014 until April 2015

TABLE 2. Total cell count and relative abundance of HAB species recorded in Marudu Bay

Phytoplankton family	Mean count (x10^3 cell 1^{-1})	%	S1 (%)	S2 (%)	S3 (%)	S4 (%)	S5 (%)	S6 (%)	S7 (%)	S8 (%)	S9 (%)	S10 (%)
Bacillariaceae	1.23	3.1	1.2	1.0	7.3	5.9	4.1	4.1	9.0	1.7	10.0	1.6
Ceratiaceae	0.24	0.6	1.1	0.6	0.8	0.7	0.7	0.4	0.6	0.3	0.4	0.3
Chaetocerotaceae	27.58	69.8	79.5	75.3	65.5	46.4	64.2	63.2	47.0	62.9	38.2	81.4
Coscinodiscaceae	0.75	1.9	2.7	1.4	1.2	1.9	0.7	0.5	0.6	2.5	0.6	2.8
Lauderiaceae	1.06	2.7	2.2	3.9	1.1	3.8	1.2	0.5	1.1	3.7	1.3	3.6
Leptocylindraceae	0.70	1.8	1.2	3.0	1.9	1.1	2.4	1.9	3.0	2.1	3.4	0.4
Skeletomataceae	1.55	3.9	2,7	1.3	7.7	12.2	6.5	9.1	0.6	0.9	20.8	0.4
Rhizosoleniaceae	2.50	6.3	2.6	6.9	3.3	4.1	5.5	9.3	12.0	19.6	6.4	2.8
Thalassionemataceae	2.52	6.4	4.0	4.2	6.2	15.7	8.2	8.6	20.6	4.4	15.4	3.6
Other	0.88	2.2	0.7	1.6	2.5	6.1	5.0	0.8	3.1	0.8	1.2	1.9
Potential HABs	**0.51**	**1.3**	**2.0**	**0.8**	**2.5**	**2.1**	**1.5**	**1.6**	**2.4**	**1.1**	**2.3**	**1.2**
Phytoplankton mean count (x10^3 cell l^{-1})	34.9±25.6		57.7± 3.8	51.5± 2.9	32± 2.5	15± 2.2	20.9± 5.3	12± 1.2	12.1± 1.8	36± 5.6	14.2± 1.3	61.8± 5.7
HAB mean count (x10^3 cell l^{-1})	0.51±0.30		1.17± 0.09	0.48± 0.03	0.80± 0.03	0.34± 0.02	0.32± 0.03	0.21± 0.01	0.29± 0.01	0.41± 0.03	0.37± 0.03	0.76± 0.05

TABLE 3. Total cell count and relative abundance of HAB species recorded in Marudu Bay from February 2014 to January 2015

Phytoplankton family	IM			SWM			IM			NEM			IM
	Apr (%)	May (%)	June (%)	July (%)	Aug (%)	Sept (%)	Oct (%)	Nov (%)	Dec (%)	Jan (%)	Feb (%)	Mar (%)	Apr (%)
Bacillariaceae	25.0	16.5	2.2	1.5	0.1	0.3	7.1	23.8	2.3	30.6	2.8	2.4	12.5
Ceratiaceae	0.6	2.2	2.2	0.2	0.1	0.1	0.3	4.2	0.4	2.5	2.0	1.0	5.1
Chaetocerotaceae	12.1	21.4	79.0	91.6	5.7	81.8	61.7	34.2	81.8	19.3	49.5	28.9	27.7
Coscinodiscaceae	0.6	0.7	0.1	0.5	0.0	3.3	0.2	0.6	0.9	5.1	2.6	1.0	1.5
Lauderiaceae	0.0	0.0	0.0	0.0	0.0	4.6	0.4	0.0	0.6	0.7	3.8	0.0	0.4
Leptocylindraceae	1.7	1.0	2.9	0.5	0.2	1.9	3.8	6.8	0.5	4.5	1.1	0.0	1.9
Skeletomataceae	27.7	27.9	10.3	0.0	0.5	0.8	8.8	0.0	6.6	6.5	0.0	2.3	0.0
Rhizosoleniaceae	0.4	0.4	0.2	3.0	77.0	3.4	6.1	4.6	0.7	2.2	0.5	0.1	7.8
Thalassionemataceae	20.6	20.4	0.8	0.2	15.8	2.6	2.6	0.8	1.8	16.6	29.4	58.9	25.3
Other	11.1	9.0	2.1	2.5	0.6	1.2	8.7	24.2	4.4	11.4	8.0	5.0	16.7
Potential HABs	0.2	0.5	0.2	0.0	0.0	0.0	0.3	0.8	0.0	0.6	0.3	0.4	1.1
Phytoplankton mean count (x10^3 cell l^{-1})	6.0± 3.6	13.5± 11.9	54.8± 26.9	35.9± 42.2	20.4± 22.6	194,8± 20.7	21.2± 17.4	15.5± 10.6	41.2± 39.9	5.1± 3.5	14.2± 5.8	9.6± 7.5	31.73± 2.00
HAB mean count (x10^3 cell l^{-1})	132.7± 54.3	700.6± 547.2	742.7± 324.5	44.8± 48.2	34.3± 39.2	342.0± 25.4	698.7± 542.8	745.8± 500.2	156± 99.4	306.2± 153.7	469.2± 127.4	396.2± 299.1	334.7± 12.5

harmful phytoplankton in May, June, October and November 2014 (700 to 750 cells/L) was significantly higher (p<0.05), but July and August 2014 (30 to 40 cells/L) was significantly lower (p<0.05) than that in other months (160 to 470 cells/L).

SPATIAL AND TEMPORAL VARIATION OF POTENTIALLY HARMFUL PHYTOPLANKTON SPECIES IN MARUDU BAY

Result of the present study demonstrated that *D. caudata, D. miles*, *C. fursus*, *P. sigmoides, P. triestinum* and *P. micans* were distributed evenly (p>0.05) throughout the bay (Figure 5). However, cell density of *C. furca* was significantly higher (p<0.05) in station 1, while *Pseudo-nitzschia* sp. was significantly higher (p<0.05) in stations 1, 3 and 10 than that in other stations.

For temporal variations, the cell densities of *D. caudata*, *D. miles*, *P. sigmoides* and *P. triestinum* were low throughout the year. The cell density of *P. micans* was slightly higher in May 2014 and March 2015, but not statistically significant (p>0.05). The peak cell density of *C. furca* was recorded in June 2014, while the high peaks for *Pseudo-nitzschia* sp. cell density were in May, October and November 2014.

RELATIONSHIP BETWEEN ENVIRONMENTAL PARAMETERS AND NUTRIENTS WITH POTENTIALLY HARMFUL PHYTOPLANKTON IN MARUDU BAY

Five factors were extracted from a principal component analysis of 14 variables (only 12 variables contributed to the factors) (Table 4). The factors explained 34, 16, 13, 9 and 8% of the total variances, respectively. In the first factor, DIP, ammonia and DIN had significant positive loading. In the second factor, DSi, Si:N and Si:P has strong positive loading, while salinity produced significant reverse influence. In the third factor, temperature, salinity and current speed has shown to have positive effects. For the forth factor, reduced form of nitrogen (NO_3-N and NO_2-N) had significant positive loading. In the fifth factor, N:P ratio showed strong positive loading.

From the loadings of each variable on the five factors, it could be deduced that Factor 1 represented increased in DIP, DIN and NH_4-N. Factor 2 was associated with dropped in salinity, but raised in silica and its ratios. Factor 3 represented rising temperature, salinity, and current speed. Factor 4 associated with oxidized nitrogen and Factor 5 associated with raised in N:P ratio.

In general, logarithmic transformed of total phytoplankton cells density were positively associated with temperature, current speed, NH_4-N, DIN, Factors 1 and 3, whereas negatively correlated to visibility, dissolved oxygen, NO_3-N and DSi, Si:N and Si:P ratios. On the other hand, the logarithmic transformed total cells density of potentially harmful phytoplankton responded positively to temperature, DIP, NH_4-N and DIN, while negatively correlated to NO_3-N and DSi.

The Pearson's correlation test indicated that *D. caudata* associated positively to temperature, but correlated negatively to DSi and Si:N ratio. *C. furca* and *C. fursus* responded positively to NH_4-N, DIN and Factor 1, while *P. micans* and *P. sigmoides* responded positively to DIP.

DISCUSSION

ENVIRONMENTAL PARAMETERS AND NUTRIENTS

The current study recorded only a small difference in most of the water parameters throughout the sampling stations.

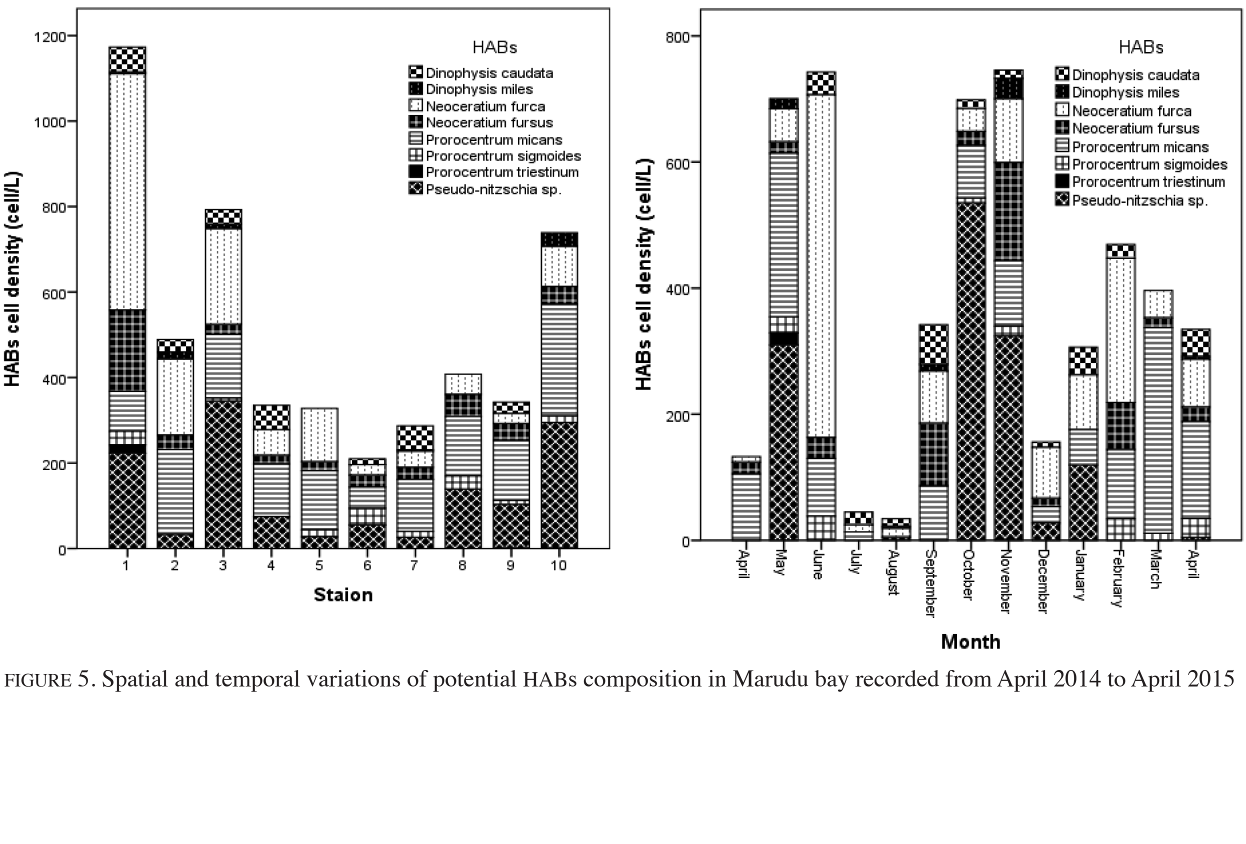


FIGURE 5. Spatial and temporal variations of potential HABs composition in Marudu bay recorded from April 2014 to April 2015

TABLE 4. Principal component analysis: Varimax rotated component matrix

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Temperature	0.036	-0.228	**0.804**	-0.086	-0.108
Salinity	0.035	**-0.555**	**0.621**	-0.072	0.126
Current Speed	0.332	-0.068	**0.754**	-0.132	-0.076
DIP (µm)	**0.809**	-0165	0.201	0.031	-0.429
NO_3 (µm)	-0.077	0.14	-0.158	0.87	-0.042
NO_2 (µm)	0.038	0.123	-0.035	0.868	-0.064
NH_4 (µm)	**0.956**	-0.122	0.11	-0.069	0.169
DSi (µm)	-0.075	**0.903**	-0.124	0.289	-0.088
DIN (µm)	**0.96**	-0.112	0.1	0.003	0.167
NP ratio	0.21	-0.073	0.159	-0.044	**0.873**
SiN ratio	-0.179	**0.769**	-0.305	-0.005	-0.244
SiP ratio	-0.165	**0.855**	-0.084	0.108	0.317
Total variation explained (%)	34	16.2	12.5	9.4	8

Significant loadings are in boldface;

TABLE 4. Pearson's correlation coefficients between biological parameters (phytoplankton and HAB) after logarithmic transformation and main environmental variables

	Total phytoplankton	Total HABs	*D. Caudata*	*Neoceratiou furca*	*N. fursus*	*P. micans*	*P. sigmoides*	*Pseudonitzschia*
Secchi depth	-0.13**	-	-	-	-	-	-	-
Temperature	0.32**	0.36**	0.637**	0.37*	-	-	-	-
pH	-	-	-	-	-	-	0.55*	-
Salinity	-	-	-	-	-	-	-	-
DO	-0.20**	-	-	-	-	-	-	-
DIP	-	0.25**	-	-	-	0.36**	-	-
Current	0.29**	-	-	-	-	-	-	-
NO_2-N	-	-	-	-	-	-	-	-
NO_3-N	-0.20**	-0.20**	-	-	-	-	-	-
NH4-N	0.31**	0.30**	-	0.46**	0.53**	-	-	-
DSi	-0.26**	-0.16*	-0.63**	-	-	-	-	-
DIN	0.10**	0.28**	-	0.45**	0.51**	-	-	-
N:P	-	-	-	-	-	-	-	-
Si:N	-0.11**	-	-0.61**	-	-	-	-	-
Si:P	-0.12**	-	-	-	-	-	-	-
F1	0.37**	0.40**	-	0.57**	064**	-	-	-
F2	-	-	-	-	-	-	-	-
F3	0.23**	-	-	-	-	-	-	-
F4	-	-	-	-	-	-	-	-
F5	-	-	-	-	-	-	-	-

The significant coefficients:**, p<0.01; *, p<0.05.

The insignificant spatial variation of these parameters is most likely due to the small sampling area covered in current study. In addition, the low water turbidity in stations 1, 2, 3, 4 and 10 was mainly due to shallower water in bay pocket.

Temporal variations of rainfall and environmental parameters in Marudu Bay are determined by the seasonal wind regime and by local topography. During NEM, the wind carries the moist air from the South China Sea, thus bringing high rainfall in northern coast of Sabah (Malaysian Meteorology Department 2014). In general, water temperature, salinity and visibility were relatively lower in NEM than that in SWM. This is because the heavy rainfall brings suspended particles to study area by river run-off and also reduced the water salinity by diluting effect. Similar observation has been reported, where heavy rainfall during monsoon resulting in high water turbidity due to rivers and land run-offs (Adam et al. 2011; Buyukates & Roelke 2005; Jalal et al. 2011; Mohammad-Noor et al. 2012). However, the current speed was significantly lower in NEM despite the higher mean wind speed in NEM. This is because Marudu Bay is sheltered by Banggi Island from the strong water current that generated by strong winds from the northeast (Azaza et al. 2008). During SWM, Marudu Bay experiences less precipitation due to the Crocker Range Mountains blocking humid wind from reaching Marudu Bay. The relatively dried wind from the southwest generates strong current in Marudu Bay and thus resulting in high water turbidity and high DO concentration.

Based on DIN and DIP concentrations (1.5 and 0.2 µM, respectively) in Marudu Bay, the environment of Marudu Bay was comparable to the characteristic of mesotrophic water body, which implies the nutrients in the bay were depleted most of the time (Hakanson & Blenckner 2008; Ludberg et al. 2005). The temporal variation of nutrients concentration in current study was highly fluctuated. This is because the nutrient concentration is influenced by the combination of interaction from various factors including hydrodynamic, human activities, primary productivity and bacterial activities (Anderson 2009; Sany et al. 2014; Tan & Ransangan 2015).

PHYTOPLANKTON AND POTENTIALLY HARMFUL PHYTOPLANKTON

Eight potentially harmful phytoplankton species were detected in Marudu Bay. The potentially harmful phytoplankton community in Marudu Bay was dominated by *C. furca* and *P. micans*. Similar observation was reported in the east of Sabah, where *C. furca* (12.9×10^3 cells/L) and *P. micans* (5×10^3 cells/L) were the dominant potentially harmful phytoplankton community under low nutrient environments (Mohammad-Noor et al. 2012). The dinoflagellate, *Prorocentrum* sp. and *Ceratium* sp. are commonly found in Malaysian water. Blooms of *C. furca* have been reported in Penang, Malaysia and in Phuket, Thailand which associated with fish kill event (Lim et al. 2012). Moreover, water discoloration in Selat Tebrau due to high concentration of *Prorocentrum* sp. was reported in the late 2002 (Usup et al. 2008). In addition, the occurrence of *P. micans* and *C. furca* have also been reported worldwide. The blooms of *C. furca* have been reported in Japan (Fukuyo et al. 1990) and in Tolo Harbour, Hong Kong (Wong et al. 2010), while *P. micans* was reported to cause red tide in Black Sea, Turkey (Turkoglu & Koray 2004) and Japanese coastal water (Fukuyo et al. 1990).

On the other hand, the third most abundance potentially harmful phytoplankton species, *Pseudo-nitzschia* sp. in Marudu Bay could cause adverse effect to marine organisms even at low cell densities of several hundred per liter (Tan & Ransangan 2015). Several species of *Pseudo-nitzschia* have been reported to produce neurotoxin domoic acid. These species include *P. australis*, *P. kodamae, P. caliantha*, *P. cuspidata*, *P. delicatissima*, *P. fraudulenta*, *P. galaxiae*, *P. heinii*, *P. multiseries*, *P. multistriata*, *P. pungens*, *P. pseudodelicatissima, P. seriata* and *P. turgidula* (Bargu et al. 2002; Bill et al. 2005; Lundholm et al. 2002; Teng et al. 2014; Trainer et al. 2012). To date, at least four toxic or potentially toxic *Psedo-nitzschia* species (*P. pseudodelicatissima*, *P. calliantha*, *P. delicatissima* and *P. pungens*) have been confirmed to occur in the north coast of Sabah (Teng et al. 2013).

Fortunately, the two commonly reported HAB species, *Pyrodinium bahamense* var. *compressum* and *Cochlodinium polykrikoides* in the west coast of Sabah were not observed in the current study. This might due to the coastal water of Marudu Bay has much lower nutrient concentrations than that in coastal waters of west coast of Sabah (Table 3). The growth of *P. bahamense* var. *compressum* is known to be higher in nutrient enriched environments (Adam et al. 2011; Wang et al. 2008). Therefore, the absent of *P. bahamense* var. *compressum* in Marudu Bay might be explained by the low nutrient level or it could also mean that the cell abundance of this species was too low to be detected. Unlike *P. bahamense* var. *compressum*, the *C. polykrikoides* has a better tolerance to the environmental conditions and therefore, widely distributed in tropical and temperate waters (Adam et al. 2011). *C. polykrikoides* has been documented in Philippines (Azanza et al. 2008), Malaysia (Adam et al. 2011; Anton et al. 2008), northwest Atlantic (Tomas & Smayda 2008) and South Sea of Korea waters (Lee 2008). It is interesting to note that, a massive bloom of *C. polykrikoides* has occurred nearby the current study area, from Brunei Bay towards the north of west coast of Sabah before reaching Palawan, Philippines (Anton et al. 2008). Since Marudu Bay is very near to Palawan and Sepangar Bay, therefore, it is likely to occur at Marudu Bay. However, the present study did not detect the occurrence of *C. polykrikoides* in the bay. The low nutrient concentration may explain the absence of this species in the bay (Lee 2008).

The cell abundance of potentially harmful phytoplankton in the current study ranged from 102 to 842 cells/L. This value was much lower than the cell abundance of potentially harmful phytoplankton recorded at east coast (3.04×10^4 cells/L) (Mohammad-Noor et al. 2012) and west coast of Sabah (7.86×10^6 cells/L) (Adam et al. 2011) (Table 5). The low cell density of potentially harmful phytoplankton in current study might be explained by the low nutrients concentration.

SPATIAL AND TEMPORAL VARIATION OF POTENTIALLY HARMFUL PHYTOPLANKTON SPECIES IN MARUDU BAY

Cell density of *C. furca* was higher in station 1, where the NH_4-N concentration was also slightly higher than other stations, due to continue supply of sewage effluence from the nearby coastal communities. Some dinoflagellates has been reported to produce specialized extracellular enzymes for hydrolysis of organic nitrogen substrates (Palenik & Morel 1990; Tan & Ransangan 2015) and also associated with uptake of reduced form of nitrogen including ammonia and urea (Berg et al. 2003; DeYoe & Suttle 1994; Glibert et al. 2001). In the current study, development of high density of *C. furca* in June was corresponded to the drastically decreased in NH_4-N. Therefore, it is reasonable to believed that *C. furca* prefer NH_4-N as nitrogen source in NO_3-N depleted environment.

On the other hand, accumulation of *Pseudo-nitzschia* sp. in current study was observed in high silica stations and months. Since *Pseudo-nitzschia* sp. is a diatom, it require

TABLE 5. Comparison of environmental parameters and HAB cell density in the north, east and west coast of Sabah (Mean± SD)

Parameter	North coast (This study)	East coast (Mohammad-Noor et al. 2012)	West coast
Temperature (°C)	29.4 ± 1.0	29.9 ± 0.4	30.2 ± 1.1 (Adam et al. 2011)
Salinity (psu)	31.4 ± 3.1	32.7 ± 0.5	29.8 ± 2.1 (Adam et al. 2011)
pH	8.22 ± 0.3	8.1 ± 0.1	8.4 ± 1.1 (Adam et al. 2011)
Mean total No. of HAB ($x10^4$ cells l^{-1})	0.03	3.04	~786 (Adam et al. 2011)
Nitrate (μM)	1.54 ± 1.79	0.60 ± 0.31	19.87 ± 11.39 (Mohammad-Noor et al. 2014)
Phosphate (μM)	0.18 ± 0.17	1.02 ± 0.56	1.21 ± 0.82 (Mohammad-Noor et al. 2014)

silica to synthesis their external shell (Anderson et al. 2002; Tan & Ransangan 2015; Turner et al. 1998). Therefore, the high silica concentration in related stations and month could promote the growth of *Pseudo-nitzschia* sp.

RELATIONSHIP BETWEEN ENVIRONMENTAL PARAMETERS AND NUTRIENTS WITH POTENTIALLY HARMFUL PHYTOPLANKTON IN MARUDU BAY

The result of Pearson's correlation test demonstrated that warmer water, higher salinity, stronger current speed and higher nutrients concentrations (DIN, DIP, NH_4-N) promote the growth of phytoplankton and potentially harmful phytoplankton population in Marudu Bay. The finding of current study was in agreement with other studies, where the growth of phytoplankton and HABs are highly influenced by water temperature (Lim et al. 2006), salinity (Mohammad-Noor et al. 2012), nutrient (Jin et al. 2013; Smith 2006) and water turbulent (Liu et al. 2002; Maso et al. 2006). However, high turbidity, nitrate depletion, low silica concentration and its ratios frequently became the limiting factor for developing phytoplankton blooms in Marudu Bay. Similar observations have been documented in many studies where exhaustion of nitrate and silica played important roles in the termination of the blooms of phytoplankton mainly the diatom (Gaytan-Herrera et al. 2011; Turner et al. 1998). In addition, light limitation has been reported to reduce the growth of phytoplankton by the exponent to 2/3 (Mei et al. 2009).

On the other hand, different genus of potentially harmful phytoplankton responded distinctively to different environmental conditions. This is because different group of phytoplankton has different nutritional strategies (Berg et al. 2003; Smayda 1997) and has distinctive optimum environmental conditions to growth (Mohammad-Noor et al. 2012; Tan & Ransangan 2015). In general, the growth of *C. furca* and *C. fursus* mainly influenced by the availability of PO_4-P and NH_4-N, while the growth of *P. micans* and *P. sigmoides* mainly promoted by high DIP.

CONCLUSION

The current study indicated that the Marudu Bay is currently safe from the HAB problem caused by the two HAB species, *P. bahamense* var. *compressum* and *C. polykrikoides* which are commonly found in other coastal water of Sabah. However, there were eight potentially harmful phytoplankton species detected in the water of Marudu Bay. Although no paralytic shellfish poisoning incidence has so far been reported, the excessive agriculture activities taking place surrounding the upstream of rivers flowing into the bay can contribute nutrients load that may be able to trigger HAB bloom in the future if the existing management practices of agricultural activities are not improved.

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