Journal of Sustainability Science and Management
Volume 13 Number 5, 2018
ISSN: 1823-8556

EFFECT OF MONSOONAL FLUCTUATIONS ON MICRO-PHYTOPLANKTON AND CHLOROPHYLL-*a* RELATIONSHIP IN REEFS ECOSYSTEMS OF SOUTH CHINA SEA

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Abstract: Phytoplankton are a key primary producer in aquatic ecosystems and mediate energy transfer between various organisms. Herein, we correlated chlorophyll-*α* contents with spatial and temporal fluctuations of micro-phytoplankton in a tropical coral reef ecosystem of the Bidong Archipelago during southwest and northeast monsoon seasons and made comparisons of pre-season and post-season primary production. Micro-sized phytoplankton were sampled using a slow vertical hauling technique with a shallow length of 20-micron mesh net, which allows meso-sized zooplankton to escape. Chlorophyll-*α* samples were collected from surface and bottom water layers using a Niskin water sampler. Micro-phytoplankton densities and chlorophyll-*α* concentrations onset bloom was from pre-southwest monsoon, peaked during the post-southwest monsoon and fluctuated significantly through the monsoon season. Mean of chlorophyll-*α* contents from surface and bottom water layers were positively correlated with micro-phytoplankton densities at Station's 1, 7, 9 and 10. Station 7 was located at a mariculture farm and the fluctuation of primary producers differed little between sampling seasons, reflecting environmental interference but showed the strongest correlation (r=0.98) between each other. Whereas chlorophyll-*a* contents were correlated with micro-phytoplankton contents in water columns, they were not exclusively dependent on micro-phytoplankton abundance, as spatial and monsoonal factor play roles. The data suggested chlorophyll-*α* contents were indication of micro-phytoplankton distributions, and relevant to determine micro-phytoplankton biomass and nutrient status of the study area.

KEYWORDS: Chlorophyll-*α*, coral reefs, micro-phytoplankton, monsoon, South China Sea

Introduction

Phytoplankton are primary producers in ocean ecosystems and produce chlorophyll-*a* as a base food that encourages energy transfer between aquatic organisms in aquatic food chains (Boonyapiwat, 1997; Jamshidi & Abu-Bakar, 2011; Cloern *et al.,* 2014;). However, high abundance of phytoplankton does not necessarily correlate with high concentrations of chlorophyll-*a*. Phytoplankton are microscopic single-celled organisms that drift with water currents (Garrison *et al.,* 2000) and can grow to form large visible colonies. Phytoplankton have been observed as nanoplankton and microplankton with range of size between 2-20 micro meter (µm) and 20-200 µm respectively, classified broadly as microalgae and cyanobacteria (Boonyapiwat, 1997; Bajarias, 2000). Microalgae and cyanobacteria produce chlorophyll-*a* pigments to capture energy from the sunlight and produce food, signify the focus on the micro-sized phytoplankton in this study.

Chlorophyll is produced by microalgae and changes from green to blue or red due to movements of electrons. Because chlorophyll carries the bulk of the energy that is fixed during photosynthesis, concentrations of chlorophyll-*a* are an indicator of phytoplankton biomass and a proxy for nutrient status (Brando *et al.,* 2006). Studies by Cloern *et al.* (2014), Monbet (1992) and, Trigueros and Orive (2000) and suggest that tide is a crucial factor that controls phytoplankton fluctuations, because nutrient loads that are driven to coastal areas by heavy rainfall are transported to adjacent areas by tidal flows, leading to increases in chlorophyll-*a* concentrations and phytoplankton densities. Tidal mixing can conversely cause re-suspension of fine sediments, leading to increased turbidity and reduced light penetration, and hence limited chlorophyll-*a* contents. Previously, Tan *et al.* (2006) showed that upwelling under conditions of strong winds and currents during the northeast monsoon resulted in a high concentrations of chlorophyll-*a*, whereas downwelling during the southwest monsoon reduced chlorophyll-*a* concentrations.

Phytoplankton contents vary between ocean areas and between tropical to temperate regions at various times of the year (Farhadian & Pouladi, 2014). Accordingly, fluctuations of phytoplankton contents in water columns can be used as biological indicators of the entire marine environment and correlated with major environmental events (Hays *et al.,* 2005). However, knowledge of their distributions, especially in tropical reef ecosystems, remains limited to studies of phytoplankton that were performed in the Straits of Malacca (Muhammad Adlan *et al.,* 2012; Wan-Maznah *et al.,* 2016) and in mangrove (Saifullah *et al.,* 2014) and non-coral reef coastal systems (Salleh & Ruslan, 2010; Mohammad-Noor *et al.,* 2012; 2013). Thus, we determined temporal variations in phytoplankton abundance and chlorophyll-*a* concentrations in different water layers.

Phytoplankton are known to have low production rates in coral ecosystems (Sakka *et al.,* 2002), leading to challenges of data collection. Coastal waters generally contain low value of chlorophyll-*a* even in blooming period in most marine areas, but according to Trigueros and Orive (2000), enhance of resident nutrient can increase their concentration up to 100 micro-gram per liter (μgL^{-1}). As one of the study station were located and directly influenced by-product from the mariculture activity, this study aimed to spatially track the changes of these microplankton fluctuation through monsoonal pattern. The determinations of abundance and spatial distributions in ecosystems may facilitate understanding of the conditions for organisms at other trophic levels in reef ecosystems as phytoplankton production are known correlated with fisheries landing (Nixon, 1988; Cloern *et al.,* 2014). Moreover, there are a report by Taniguchi *et al.* (1997) stated that an increase of chlorophyll-*a* concentration has something to do with the increasing density of flagellates.

Materials and Methods

Sample Design

Samples were taken from 10 fixed stations Pulau Bidong and Pulau Karah islands (Bidong Archipelago), Terengganu (Latitude 5.62°N; Longitude 103.06°E), east coast of Peninsular Malaysia, Southern-South China Sea (Figure 1). We performed a vertical plankton haul technique to collect micro-phytoplankton samples in triplicate using a 20 µm mesh size nets with 30 ×30-cm square mouth openings (Budge *et al.,* 2001; Wu *et al.,* 2014). This technique was chosen as a standardized method for capturing only micro-phytoplankton in the present trophic level investigation. Accordingly, the plankton net was gently hauled from a stationary vessel at 1 meter (m) from the sea bottom up to the surface. A before and after reading by an attached mechanical flowmeter (General Oceanics model 2030R, USA) to net mouth was recorded (Harris *et al.,* 2000). Samples were fixed with 3% of formaldehyde solution, and was concentrated before undergo the Lackey's Drop Method (APHA, 1985). The density (cells per meter cubic; $cells.m^{-3}$) were in mean ± standard deviation (SD).

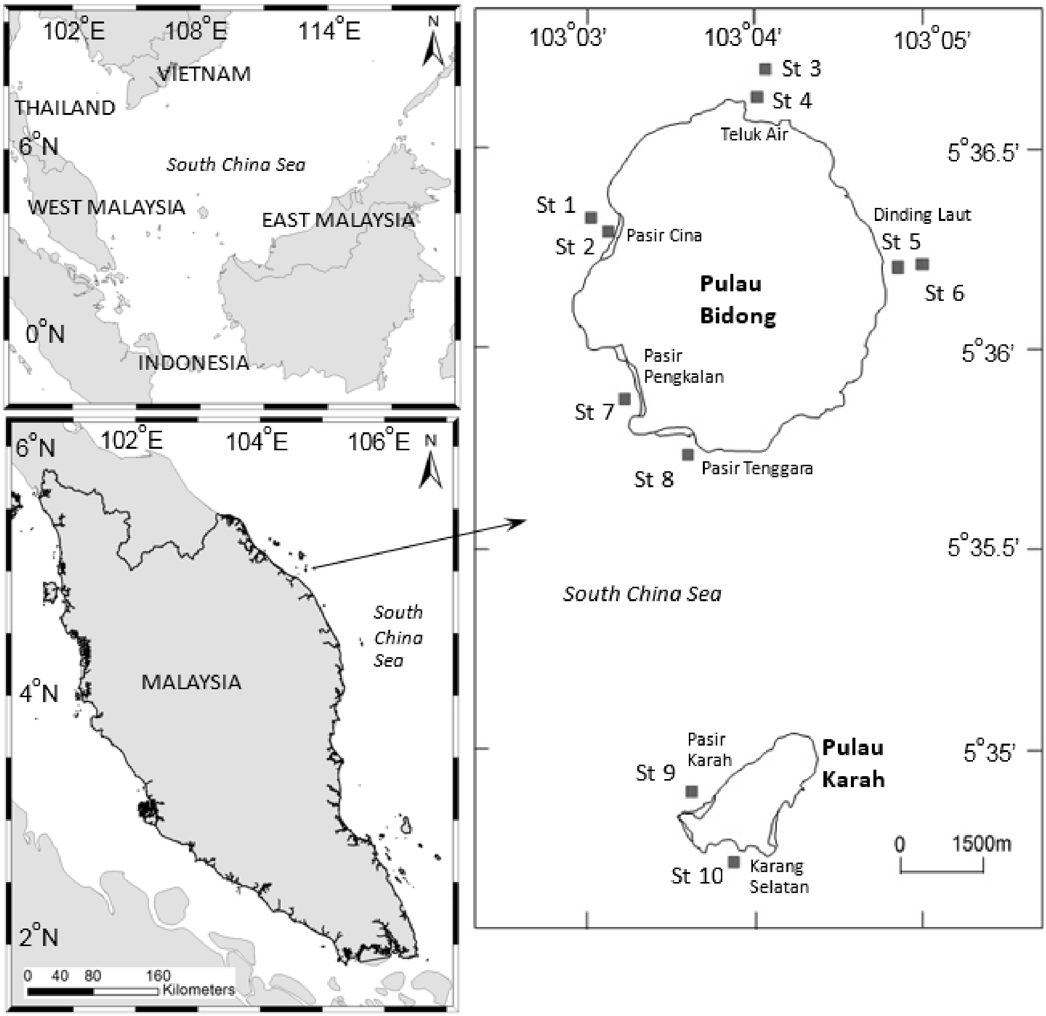

Figure: 1: Location of Malaysia and sampling stations at Pulau Bidong and Pulau Karah (Bidong Archipelago), South China Sea.

Coral reefs in the Bidong Archipelago form a tropical type reef ecosystem, and the resident organisms tolerate relatively warmer sea surface conditions throughout the year. Bidong Archipelago is located at the western side of the South China Sea, and the present sampling areas were chosen according to coral reef productivity and nutrient support from estuaries (Howarth, 1988) of the main land. Bidong Archipelago is subject to significant wind, wave, and rainfall patterns during the northeast monsoon (Daud & Akhir, 2015), which strikes annually from November until March. In comparison, the southwest monsoon season is drier and warmer (Wang *et al.,* 2006) and usually occurs from late May until September (Malaysia Meteorology Department, 2015).

Water patterns during the post-northeast (PNE) monsoon are believed to be a major factor in conserving the condition of the reef habitats (Toda *et al.,* 2007). Thus, according to monsoon seasons, pre-southwest (PreSW) samples were taken during the latter half of May 2014 (16th May), southwest (SW) samples were taken on the 08th August 2014, post-southwest (PSW) samples were taken on the 19th September 2014, pre-northeast (PreNE) were taken on the 17th October 2014 according to Yip *et al.,* (2015), northeast (NE) samples were taken on the 06th February 2015, and PNE samples were taken on 06th March 2015. Triplicate water samples for were collected at surface and bottom layer using Niskin water sampler. Samples were stored in the dark until vacuum filtering through GF/C 1.2-µm Whatmann Glass Micro-fiber filter papers, and were then stored in individual centrifuge tubes at −20°C for subsequent analyses.

Sample Analysis

Chlorophyll-*a* samples were ground and thoroughly mixed using a glass rod in 10 milliliters (ml) aliquots of 90% acetone. This solvent was previously optimized for extraction efficiency and integrity of isolated chlorophyll pigments (Mantoura & Llewellyn, 1983). Moreover, to further minimize chlorophyll degradation, samples were wrapped in aluminum foil and were stored in the dark for as long as possible, as described previously (Lenz & Fritsche, 1980). After 24-hours storage in a freezer, samples were homogenized and then centrifuged at 2000 rotor-per-minute (rpm) for 10 minutes. Samples were then transferred into 1-centimeter (cm) cuvettes using a Pasteur pipette and absorbance was determined at wavelengths of 750, 665, 663, 645 and 630 nanometer (nm) using an Ultraviolet-Visible Spectrophotometer (Shimadzu UV-1800, Japan). Chlorophyll-*a* concentrations were calculated according to the equation described by Strickland and Parsons (1972). Mean density of micro-phytoplankton was compared among monsoonal season by one-way ANOVA, followed by post hoc test (Tukey). The same goes for chlorophyll-*a*. Relationships between the distributions of micro-phytoplankton and chlorophyll contents were identified according to Pearson's correlations. All statistical including two-way ANOVA were analyzed using MINITAB savvy, and were considered significant when $p < 0.05$. The linear regression analysis was run using Microsoft Excel to investigate and model the relationship pattern between the primary producers.

Results and Discussion

Observation under microscope showed that collection was predominant by micro-phytoplankton community. Onset of micro-phytoplankton bloom was from the PreSW monsoon, increasing to maximum during PSW monsoon season (Figure 2) apart from station (St.) 4 and St. 7. Compared to the study by Booyapiwat (1997), phytoplankton was found higher during pre-monsoon season (Sept. - Oct.) compared to post monsoon (April- May). However, specific data to the east coastal waters of Malaysia showed that phytoplankton was highest during post monsoon season. The abundance pattern may vary depend on the different area. As a study by Mohammad-Noor *et al.* (2013), phytoplankton was found at peaked during NE monsoon (December) in the coastal area of Kuantan, Pahang. A study by Sidik *et al.* (2008) found phytoplankton was highest during SW monsoon (July- Sept.) and a literally high abundance was noticed during inter-monsoon (Oct.) compared to NE monsoon (Nov.- Jan.) in cage culture area of Sepanggar Bay, Sabah, which relatively has a similar phytoplankton fluctuation to this study. Occurrence of highest abundance of micro-phytoplankton during PSW monsoon may because the coastal current flow that drive in flushed nutrients of the main land. As stated by Daud and Akhir (2015), current flow that directed to north-eastward direction to Bidong Archipelago during SW monsoon were believed transported nutrients from mangrove area of Sungai Merang to the adjacent area with the help of wind and tides (Monbet, 1992; Trigueros & Orive, 2000; Cloern *et al.,* 2014). Thus, nutrient loaded to surrounding area has increase phytoplankton production and their density (Trigueros & Orive, 2000) to maximum during the next season, PSW monsoon.

Concentrations of chlorophyll-*a* were also increased from the PreSW monsoon season and were highest during the PSW monsoon at all sampling stations except for St. 7, where chlorophyll-*a* concentrations were relatively similar at all sampling time points (Figure 2 g). However, the distribution between season in the station was analyzed significantly different ($p<0.05$; Table 1), specifically between PreSW and NE monsoon, and PreNE with NE monsoon as portrayed by Tukey's (Table 2). Chlorophyll-*a* concentrations fluctuate naturally between seasons and theoretically reflect the presence of phytoplankton in water columns (Jamshidi & Abu-Bakar, 2011; Wang et al., 2006). The SW monsoon season is generally drier and warmer on the east coast of Malaysia (MMD, 2015) providing favorable conditions for diatom growth (Muhammad-Adlan *et al.,* 2012; Colak-Sabanci, 2014; Petrou *et al.,* 2016). In the study by Tan *et al.* (2006) ambient chlorophyll-*a* concentrations increased during the NE monsoon in the Straits of Malacca, and as observed in the Bidong Archipelago, were correlated with upwelling due to wind flow. Study by Petrou *et al.,* (2016) also stated an enhanced phytoplankton production may related to the increasing upwelling, but with the mix of ocean stratification. These observations indicate that monsoon-mediated environmental changes control fluctuations in the growth of organisms, and in ecosystem dynamics.

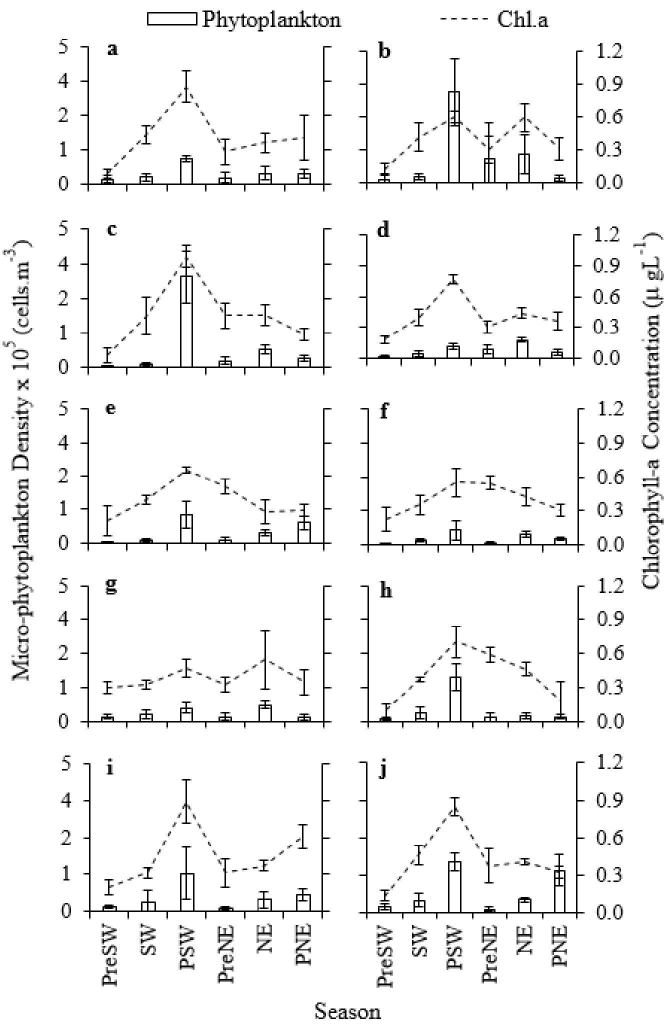

Figure 2: Temporal distributions of micro-phytoplankton (vertical bar; left axis) and chlorophyll-*a* concentrations (dotted line; right axis) at (a) St. 1, (b) St. 2, (c) St. 3, (d) St. 4, (e) St. 5, (f) St. 6, (g) St. 7, (h) St. 8, (i) St. 9, and (j) St. 10 (means ± SD); n = 3.

The highest micro-phytoplankton density was observed at St. 2, and was 331,766 ± 123,173 cells.m^{-3} (Figure 2 b) thus, only statistically significant to micro-phytoplankton density from St. 1 (Tukey's; Table 2). Micro-phytoplankton density in St.3 was also relatively high during the season, matched with the highest peak chlorophyll-*a* concentration (0.96 ± 0.09 milligram per liter (µgL^{-1}); Figure 2 c). Both were observed during PSW monsoon with agreed to Tukey test, the best monsoon season for plankton bloom (99.54% and 99.43% individual confidence level respectively). Although the sampling stations received current influences from the same direction, condition of the station itself also play role. Compared to the St. 3, St. 2 has *'Acropora'* branching coral reefs bed that recognized more effective in trapping nutrients (Wild *et al.,* 2004). Moreover, St. 2 is in a bay-shaped area, portrays the effectiveness as nutrient container. In addition, St. 2 has nutrient input from activities on the research station. However, micro-phytoplankton relationship with chlorophyll-*a* in St. 3 was significantly strong (p<0.05; R^2= 0.8353) compared to St. 2 (Figure 3). Besides nutrient perturbation that shaping the phytoplankton abundance (Badosa *et al.* 2007), pressure of predator is also believed to control density of phytoplankton in sampling area (Takahashi & Uchiyama, 2008). The study by Van de Poll *et al.* (2013) also stated sea surface temperature is more important locator factor for phytoplankton distribution compared to deeper nutrient-rich water.

Table 1: Significance level from ANOVA analysis for monsoonal sampling of primary producers in the Bidong Archipelago of the South China Sea. * p<0.05; ** p<0.01; *** p<0.001; ns, not significant

Analysis	Category	Micro-phytoplankton	Chlorophyll-*a*
1 Way ANOVA	St. 1	*	**
	St. 2	***	**
	St. 3	***	***
	St. 4	ns	***
	St. 5	*	**
	St. 6	*	*
	St. 7	*	**
	St. 8	***	***
	St. 9	**	*
	St. 10	*	***
	All	***	***
2 Way ANOVA	Season	***	***
	Station	***	***
	Interaction	***	***

The present data show that micro-phytoplankton contents and chlorophyll-*a* spatial variations were dependent on monsoon season (p<0.05) (2 Way ANOVA; Table 1). Similarly, study by Colak-Sabanci (2014) showed that increased chlorophyll-*a* concentrations followed significant growth of algae and that low phytoplankton abundance resulted in low concentrations of chlorophyll-*a*. This was supported by a strong Pearson's correlation analyses (p<0.01; Table 3) between both primary producers. Moreover, increased light penetration into the water column during dry and clear SW monsoon periods was reportedly correlated with chlorophyll-*a* concentrations, indicating significant effects on the growth of phytoplankton (Khalil *et al.,* 2009; Jamshidi & Abu-Bakar, 2011). Similarly, Ara *et al.* (2011) showed significant correlations between chlorophyll-*a* concentrations micro-size (> 20 µm) phytoplankton counts.

Table 2: Post-hoc Tukey analysis of 1 way ANOVA (MP, micro-phytoplankton; Chl-*a*, chlorophyll-*a*). * p<0.05; ** p<0.01; ns, not significant.

Category		***p*-value**		**Tukey's Significantly Different**	
		MP	**Chl-*a***	**MP**	**Chl-*a***
Station	St. 1	**	**	PSW, PreSW, PreNE	Each Season
	St. 2	ns	**	Each Season	Each Season
	St. 3	ns	**	Each Season	Each Season
	St. 4	*	**	Each Season	NE, PSW, PreSW
	St. 5	ns	**	Each Season	PSW, SW, PreNE, PreSW
	St. 6	*	**	Each Season	PSW, PreNE, PreSW
	St. 7	*	**	Each Season	NE, PreSW, PreNE, PNE
	St. 8	ns	**	Each Season	Each Season
	St. 9	*	**	PSW, SW, PreSW, PreNE	PSW, PreSW, PreNE
	St. 10	*	**	Each Season	Each Season
Season		***	***	PSW, NE,PreSW	PSW, NE, PreSW
Station		***	***	St.2, St.8, St.5, St.1, St.4, St.7, St.6	St.2, St.6

Table 3: Pearson's correlations, r between temporal micro-phytoplankton densities and chlorophyll-*a* contents at each sampling station in the Bidong Archipelago.
* p<0.05; ** p<0.01; ns, not significant.

Station	**r**	**Station**	**r**
St. 1	0.870*	St. 6	0.607^{ns}
St. 2	0.710^{ns}	St. 7	0.928**
St. 3	0.914^{ns}	St. 8	0.680^{ns}
St. 4	0.550^{ns}	St. 9	0.873*
St. 5	0.714^{ns}	St. 10	0.909*
All	0.664**		

In the present study, data from the St. 7 were inconsistent with those from the other stations, potentially reflecting the presence of anthropogenic activities in the area and differences in nutrient supply, which have previously been associated with severe damage to marine environments (Jamshidi & Abu-Bakar, 2011; Cloern *et al.,* 2014). Station 7 is located in a large scale mariculture area and was also influenced by vessel activity due to the presence of a concrete jetty (Figure 1). Hence, pollution, including waste products from fish-farming, likely accumulates in the area due to the coral reef bed and bay shape, which prevent effective transport by tide. Moreover, wind and tide directions during the SW monsoon season (Daud & Akhir, 2015) favor retention of nutrients because the location is geographically protected from NE wind flows. Alternatively, Laili and Parsons (1993) suggested that solar radiation and availability of essential nutrients that were dominant regulators of primary production in the sea (Trigueros & Orive; 2000; Cloern *et al.,* 2014).

Herein, we used chlorophyll-*a* concentrations as an indicator of nutrient status in the Bidong Archipelago, as described by Brando *et al.* (2006). Our data show that micro-phytoplankton density is strongly correlated with chlorophyll-*a* concentrations at St. 7 (r=0.998; p<0.01; Table 3), as well as portrayed by linear regression (R^2=0.8608; p<0.05; Figure 3). These results suggest that available nutrients are suspended toward the sea bottom, where productivity of primary producers is greatest. In agreement, Wang *et al.* (2006) showed that fluctuations of nutrient availability strongly influence community structures of primary producers. Transportation of nutrient was believed weakening to reach St. 4 which located far outside from the coral reefs bed. Accordingly, the micro-phytoplankton density varied similarly within the monsoonal season (p>0.05; Table 1). However, St. 10 significantly showed strong relationship between both primary producers (p<0.05; r=0.909; R^2=0.8271), was believed received nutrient support carried by wind and coastal current from main land. Due to its location relative to SW and NE monsoon wind and current directions, St. 6 may receive less nutrient influx than the other sites, potentially explaining weak relationship between chlorophyll-*a* and micro-phytoplankton as portrayed in Figure 3. Besides, low regression correlation at St. 4 and St. 8 also explain their inadequacy of nutrient, accordingly to their less-supportive location.

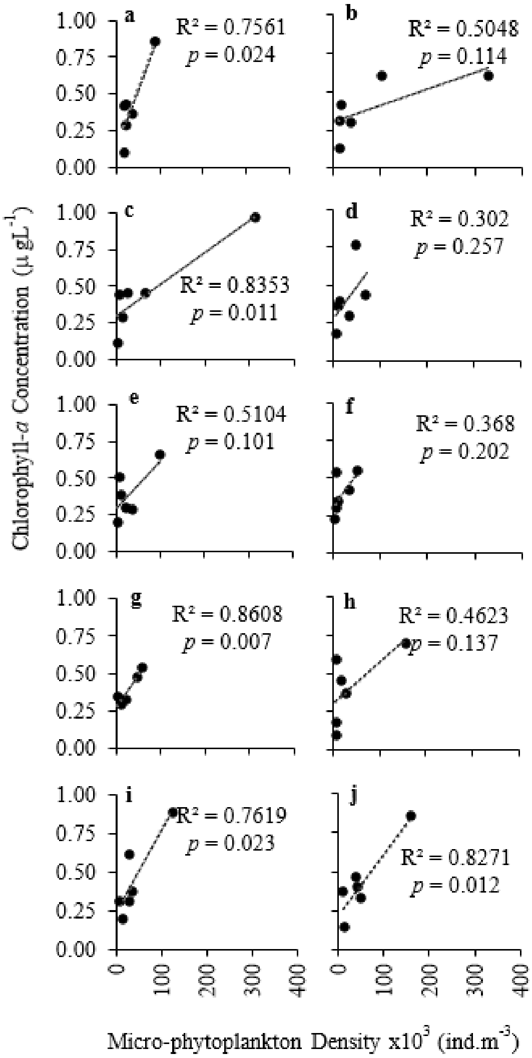

Figure 3: Linear regression (R^2) between micro-phytoplankton (ind.m-3; x axis) and chlorophyll-*a* concentrations (μgL^{-1}; y axis) at (a) St. 1, (b) St. 2, (c) St. 3, (d) St. 4, (e) St. 5, (f) St. 6, (g) St. 7, (h) St. 8, (i) St. 9, and (j) St. 10.

Conclusion

Compositions of water columns were driven by monsoon seasons and played key roles in determining micro-phytoplankton abundance and chlorophyll-*a* concentrations in tropical reef areas of the Bidong Archipelago. Critically, chlorophyll-*a* concentrations in the present water columns were significantly associated with micro-phytoplankton contents, but spatially influenced by various factors such as nutrient availability and predation on micro-phytoplankton.

Acknowledgments

The work in this report was partially funded by Higher Institution Centre of Excellence (HICoE) Research Program (Grant No. 66928) at the Institute of Oceanography and Environment (INOS), Universiti Malaysia Terengganu (UMT). The authors thank to Central Laboratory UMT, Nur 'Aliah binti Adam, Mohd Azlan bin Mohd Putra, Mohamad Afifullah bin Ahmad, Hairul Masrini binti Muhammad, and Jabatan Pengairan dan Saliran (JPS) Ampang for their contribution in this study. Thanks to Prof. David Sheehan (Khalifa University, UAE) for the improvement on the manuscript. The authors would also like to thank Enago (www.enago.com) for the English language review.

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