

***Skeletonema costatum* of mangrove ecosystem: its dynamics across physico-chemical parameters variability**

¹Nurul Shahida Redzuan, ²Pozi Milow

¹ School of Marine and Environmental Science, University Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia; ² Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia. Corresponding author: N. S. Redzuan, nurulshahida@umt.edu.my

Abstract. As part of a monitoring programme, twelve months total phytoplankton sampling were carried out starting from April 2009 to March 2010 in Carey Island mangrove ecosystem across measured physico-chemical and total phytoplankton diversity parameters. Across the one year sampling period, we found three major occurrences of mono-species bloom of *Skeletonema costatum* in the month of April, May and August 2009, in which the water body at study site displayed dark brown colour. This diatom species that displayed significant temporal variation has been found to grow rapidly when the nitrate concentration and temperature of the water column were $> 0.6 \text{ mg L}^{-1}$ and $> 31^{\circ}\text{C}$, respectively. This study shows that *S. costatum* displayed negative correlation with dissolved oxygen in the water column. Increase in *S. costatum* cells abundance at the study site proved to destabilize total phytoplankton diversity by decreasing the equitability (measured by evenness) of total phytoplankton.

Key Words: *Skeletonema costatum*, total phytoplankton, physico-chemical parameters, mono-species bloom.

Introduction. *Skeletonema costatum* (Greville) Cleve is a chain forming and a cosmopolitan diatom species that is well-known for its unique hallmark, the intercalary processes that joint and integrate two valves forming a suture line (Hasle & Syvertsen 1997). Therefore, it is really hard to confuse this species with other phytoplankton species. The species has been the subject of many studies due to its importance in aquaculture (Shamsudin 1992; Hashimoto et al 2008), its affinity towards wide spectrum of nutrients (Nayar et al 2005; Tantanasarit et al 2013; Shaik et al 2015) and also frequently reported as the dominant phytoplankton species in various ecosystems (Aké-Castillo & Vázquez 2008; Rajkumar et al 2009; Lim et al 2014).

S. costatum abilities, in tolerating high nutrients level such as the ammonium (Smayda 2004) and nitrate (Li et al 2009), and in surviving the wide range of temperature and salinity (Ebrahimi & Salarzadeh 2016) explain the species potential in causing harmful bloom worldwide. Often, the outbreaks of this monospecific bloom lead to fish kills (Huo & Shu 2005; Li et al 2009) and nutrient imbalance (Zhou et al 2017) in the water column. Heavy rainfall initiates the increase of nutrients stimulates the rapid proliferation of *S. costatum* (Li et al 2009). Similarly to the effect of heavy rainfall, prolong and continuous rainfall although decrease the nutrients concentration in the water column also proven to stimulate the rapid growth of the species (Vasudevan et al 2014). Both findings supported that *S. costatum* is an opportunistic species which can survive and utilize both the maximum and the minimum level of some nutrients in the water column.

The present study was not exclusively carried out to study the *S. costatum* species but was to monitor the temporal variability of total phytoplankton occurrence in the mangrove ecosystem. The three events of *S. costatum* blooms that were recorded and observed at our study site during total phytoplankton monitoring have somehow

called for this report to also be written and shared. With regards that this harmful *S. costatum* has been reported as the dominant species recorded in the coastlines of Malaysia (Yasin & Razak 1999; Mohammad et al 2013; Razali et al 2015), this present work aims to investigate the potential nutrients and physical parameters that stimulate the rapid growth of *S. costatum*. As part of sustainable monitoring of the ecosystem, this work also aims to investigate any significant effect of this harmful species on the diversity of the total phytoplankton. Information gained from this present study is crucial to increase knowledge on the bloom of *S. costatum* specifically in mangrove ecosystem and generally in estuarine and coastal ecosystems. In addition, the record on these bloom events can also be used for future reference in mangrove management.

Mangrove ecosystem of Langat River in Carey Island was chosen as study site with respect that it receives huge amount of nutrient from the domestic, industrial discharges and is well defined by the active aquaculture (shrimp farming) along it (Lassen et al 2004). The Carey Island mangrove lies in the vicinity of Sime Darby Oil Palm Plantation. Therefore, this present study was also a collaboration with Sime Darby Plantation that concerns on the effect of the oil palm plantation related activities on the mangrove ecosystem. The tidal activity that actively allows the exchange of the water between the Carey Island mangrove ecosystem with the Langat River (Juahir et al 2011) makes these two ecosystems interconnected. Any activities that lead to shift in the study site's water quality potentially affect the water quality of Langat River and further, the adjacent coast line.

Material and Method

Study site. Carey Island area has a humid tropical climate throughout the year with average rainfall of below 2,000 mm. The driest month is in January (120 mm) and the wettest month is in April (280 mm) (Salleh & Tajuddin 2006). The Island is listed by the Malaysian Wetland Working Group as a mangrove island, since it is mostly surrounded by mangrove. Influx of water from Sungai Langat that flows into the mangrove ecosystem making the water becomes brackish and slightly acidic. Three sampling stations were chosen and named ST1, ST2 and ST3 (Figure 1). The three stations were chosen based on their different location to river mouth and displayed significant salinity gradient. ST1 is located upstream near to a shrimp farm, ST2 adjacent to oil palm processing plant and ST3 is located downstream towards the river mouth.

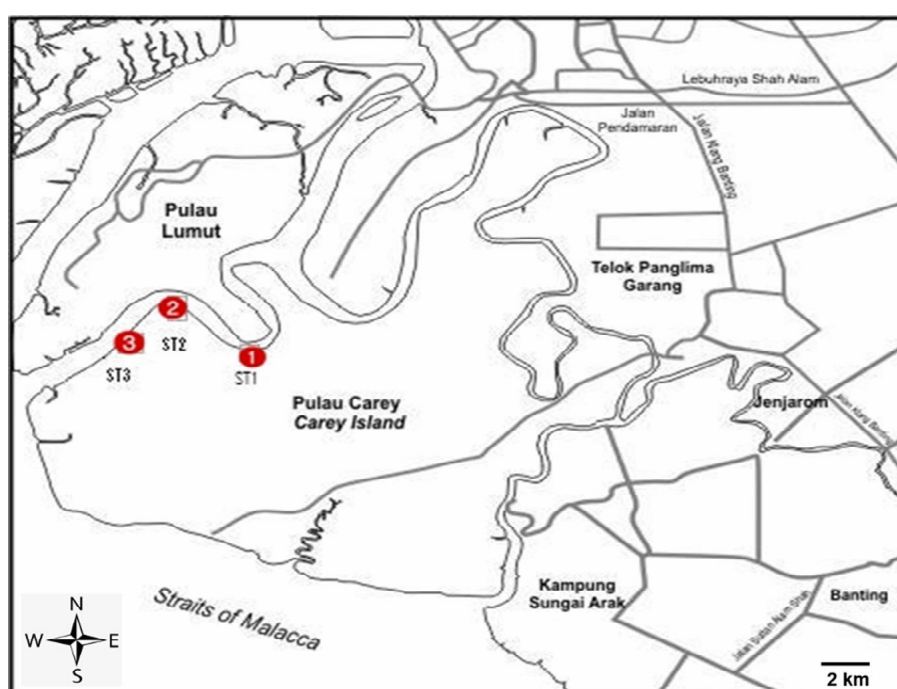


Figure 1. Map of Pulau Carey or Carey Island and study sites (ST1, ST2 and ST3).

Phytoplankton sampling, enumeration and identification. Monthly triplicates phytoplankton samples were collected using a 30 µm plankton net (attached with flow meter at the mouth to determine the volume of water passed through it) at each of sampling stations, from April 2009 to March 2010. Use of 20 µm was compromised by possibly high suspended solid (based on observation) in the water column of the study site which has caused major tear of two plankton nets. The samples were preserved in vials using 4% formalin for identification and enumeration procedures. All of the samples from the three different stations however were pooled because of their dependency (n = 9 for each variables).

One mL of preserved phytoplankton samples was added into Utermoehl sedimentation chamber followed by a drop of Lugol's iodine. The sedimentation slides were prepared following the manuals of Bellinger & Sigeo (2010). Phytoplankton were enumerated using the method described by Evans (1972) under 40x and 100x magnification of inverted light microscopy equipped with eyepiece graticules and were expressed in cells L⁻¹.

Slides prepared for enumeration were also used for phytoplankton identification since the enumeration was carried out according to the identified phytoplankton species. Cells were identified to their lowest taxonomic level, using two taxonomic keys of Hasle & Syvertsen (1997) and Salleh & Tajuddin (2006). Scanning electron microscope (SEM) was used to document and further confirm the *S. costatum* species. Samples for SEM were cleaned following the acid washing procedure by Underwood (1994).

Total phytoplankton diversity. Phytoplankton community diversity was investigated by means of its evenness (*E*) and Shannon Diversity Index (\log_2) (*H'*). Both values were calculated using Multivariate Statistical Package (MVSP) (Kovach 1999). Relative density (RD) of each of the phytoplankton species were calculated to represent the percentage of each species total density as opposed to the total phytoplankton density in the whole twelve sampling months.

Water sampling for physico-chemical parameters measurement. Water samples for chemical analyses were collected using Ruttner sampler. The samples were then transferred into polythene bottles (500 mL) for phosphate (PO₄⁻³), nitrate (NO₃⁻²), and silicate (SiO₃) analyses in the laboratory. Powder Pillow HACH reagents that were exclusive for each of the nutrients were used to measure the chemical concentrations. The procedure to measure the nutrients were carried out using Spectrophotometer DR4000 HACH and the nutrient concentrations were expressed in mg L⁻¹. YSI Model 556 MPS was used in order to measure dissolved oxygen (DO) concentration (mg L⁻¹), temperature (°C) and salinity (ppt) of water at study sites. All of the parameters were measured *in-situ*.

Statistical analyses. Spatial (between stations) and temporal (between months) variability of *S. costatum* cells abundance, total phytoplankton diversity and physico-chemical parameters of the water column were tested using one way ANOVA, with aid from IBM SPSS Statistics 25 (2017).

Results and Discussion. Two measured physico-chemical parameters in the water column, the silicate (Figure 2C) and DO (Figure 2E) concentrations were the only variables that did not significantly vary at annual scale between sampling months. Nitrate was the mostly varied parameter with the *F* value of 16.481 (*p* < 0.001) with the highest recorded concentration in April 2009 (1.17±0.06 mg L⁻¹), whereas the lowest was in June 2009 with the reading of 0.15±0.09 mg L⁻¹ (Figure 2A). Phosphate concentrations showed lower temporal variability than the nitrate. The phosphate concentration varied significantly at *p* < 0.01 with *F* value of 3.491. Water temperature at study site ranged between 28 and 31.5°C over a year. High water temperature of more than 31°C were recorded in April, May, June and August 2009 (Figure 2D). Concentration of DO in the water column although was not varied temporally, was fluctuated in range of 1.8 to 5.37 mg L⁻¹ (Figure 2E). Month of April 2009 recorded the lowest level with only 1.8±0.4 mg

of DO per litre of water. The value increased steeply in August 2009 to $5.37 \pm 1.51 \text{ mg L}^{-1}$, making it the highest DO recorded in the 12 months sampling period. Levels of water column's salinity displayed a quite uniform pattern, with significantly higher reading in May ($28.62 \pm 0.06 \text{ ppt}$) and June 2009 ($28.76 \pm 0.14 \text{ ppt}$) (both at $p < 0.001$) than other months (Figure 2F).

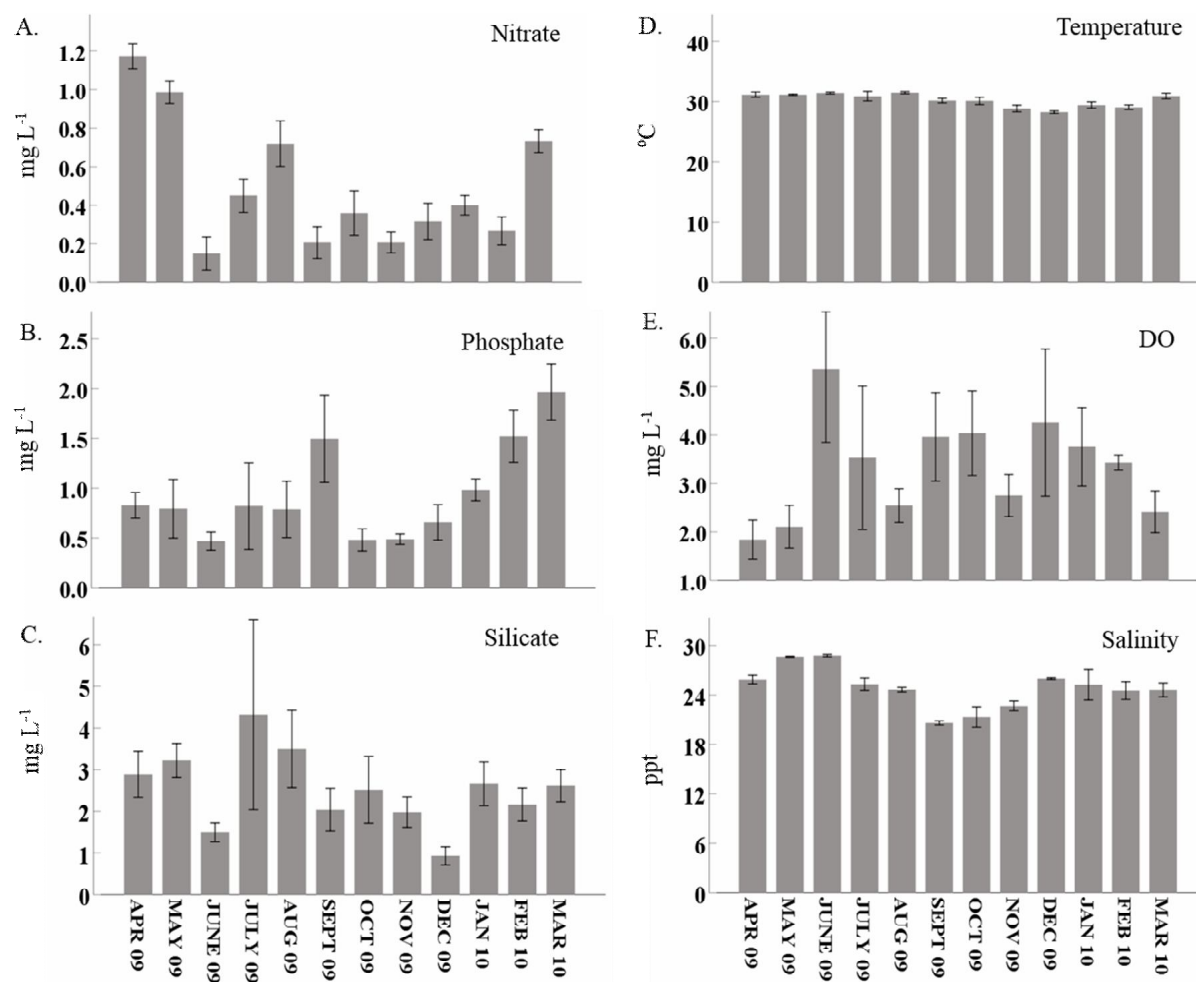


Figure 2. Temporal variability of the measured physico-chemical parameters. Values are the mean \pm SE (n = 9).

A total of 84 phytoplankton species were recorded at study site during the twelve months sampling period. With 79 recorded species were from division Bacillariophyta or the diatoms, 3 species of division Chlorophyta and 1 species recorded from both Pyrrophyta and Cyanobacteria. The relative density of the phytoplankton species was in the range of 0.03 to 49.23% with the *Peridinium cinctum* and *S. costatum* recorded with the lowest and highest values, respectively (Table 1). The high temporal RD of *S. costatum* must be caused by their high cell density in the water column during the bloom events in April, May and August 2009 and also their dominance in most of the sampling months. *Oscillatoria tenuis*, *Rhizoclonium* sp., *Chaetoceros curvisetus*, *Lauderia annulata*, *Pseudonitzschia* sp., *Pinnularia acuminata* and *Rhizoclonium* sp. were also observed to have relatively high cell density along with *S. costatum* (Table 1).

Table 1

Phytoplankton species recorded in Carey Island mangrove. RD indicates the relative density of each species across the twelve months of sampling period. Note that the authority of each species is not included in this table

No.	Species	RD (%)	No.	Species	RD (%)
	Division Bacillariophyta				
1	<i>Amphora quadrata</i>	0.21	41	<i>Entomoneis alata</i>	0.19
2	<i>Asterionellopsis glacialis</i>	0.77	42	<i>Fragilaria</i> sp.	0.06
3	<i>Bacillaria paxillifera</i>	0.09	43	<i>Frustulia vulgaris</i>	0.05
4	<i>Bacteriastrium comosum</i>	0.45	44	<i>Guinardia flaccida</i>	0.14
5	<i>Bacteriastrium delicatulum</i>	0.18	45	<i>Gyrosigma scalproides</i>	0.06
6	<i>Bacteriastrium hyalinum</i>	0.39	46	<i>Gyrosigma spencerii</i>	0.06
7	<i>Bellerochea horologicalis</i>	0.78	47	<i>Lauderia annulata</i>	1.53
8	<i>Odontella longicruris</i>	0.16	48	<i>Leptocylindrus danicus</i>	1.27
9	<i>Odontella mobiliensis</i>	0.29	49	<i>Mastogloia smithii</i>	0.11
10	<i>Coronia deamilliana</i>	1.13	50	<i>Melosira moniliformis</i>	1.36
11	<i>Chaetoceros constrictum</i>	0.57	51	<i>Melosira nummuloides</i>	1.38
12	<i>Chaetoceros constrictus</i>	0.39	52	<i>Navicula peticolasii</i>	0.11
13	<i>Chaetoceros curvisetus</i>	3.63	53	<i>Navicula radiosa</i>	0.30
14	<i>Chaetoceros debilis</i>	0.21	54	<i>Nitzschia acicularis</i>	0.08
15	<i>Chaetoceros decipiens</i>	0.41	55	<i>Nitzschia longissima</i>	0.04
16	<i>Chaetoceros delicatulus</i>	0.12	56	<i>Pseudo-nitzschia</i> sp.	4.85
17	<i>Chaetoceros dichchaeta</i>	0.22	57	<i>Pinnularia acuminata</i>	5.19
18	<i>Chaetoceros laciniosus</i>	0.21	58	<i>Pinnularia</i> sp.	0.49
19	<i>Chaetoceros lauderi</i>	0.52	59	<i>Pinnularia tabellaria</i>	3.29
20	<i>Chaetoceros lorenzianus</i>	0.39	60	<i>Planktoniella sol</i>	0.13
21	<i>Chaetoceros neglectus</i>	0.04	61	<i>Pleurosigma angulatum</i>	0.09
22	<i>Chaetoceros gelidus</i>	0.60	62	<i>Pleurosigma directum</i>	0.11
23	<i>Chaetoceros</i> sp.1	0.18	63	<i>Pleurosigma elongatum</i>	0.17
24	<i>Chaetoceros</i> sp.2	0.04	64	<i>Pleurosigma</i> sp.1	0.05
25	<i>Chaetoceros</i> sp.3	0.04	65	<i>Pseudo-nitzschia cuspidata</i>	0.07
26	<i>Chaetoceros subtilis</i>	0.23	66	<i>Rhizosolenia alata</i>	0.19
27	<i>Chaetoceros tenuissimus</i>	0.13	67	<i>Rhizosolenia hebetata</i>	0.18
28	<i>Corethron criophilum</i>	0.11	68	<i>Rhizosolenia imbricata</i>	0.20
29	<i>Coscinodiscus asteromphalus</i>	0.15	69	<i>Rhizosolenia setigera</i>	0.17
30	<i>Coscinodiscus centralis</i>	0.18	70	<i>Rhizosolenia striata</i>	0.15
31	<i>Coscinodiscus concinnus</i>	0.39	71	<i>Skeletonema costatum</i>	49.23
32	<i>Coscinodiscus gigas</i>	0.08	72	<i>Stauroneis obtusa</i>	0.10
33	<i>Coscinodiscus rothii</i>	0.09	73	<i>Stauroneis pusillum</i>	0.07
34	<i>Coscinodiscus</i> sp.4	0.12	74	<i>Staurosirella pinnata</i>	0.07
35	<i>Coscinodiscus</i> sp.5	0.12	75	<i>Thalassira leptopus</i>	0.29
36	<i>Coscinodiscus subtilis</i>	0.13	76	<i>Thalassionema nitzschoides</i>	0.07
37	<i>Cyclotella meneghiana</i>	0.06	77	<i>Thalassionema javanicum</i>	1.27
38	<i>Cymbella australica</i>	0.68	78	<i>Triceratium favus</i>	0.15
39	<i>Diatoma elongata</i>	0.10	79	<i>Triceratium</i> sp.	0.14
40	<i>Ditylum brightwelli</i>	0.20			
	Division Chlorophyta			Division Pyrrophyta	
80	<i>Mougeotia</i> sp.	0.25	83	<i>Peridinium cinctum</i>	0.03
81	<i>Cosmarium humile</i>	0.07		Division: Cyanobacteria	
82	<i>Rhizoclonium</i> sp.	5.40	84	<i>Oscillatoria tenuissima</i>	6.06

S. costatum that displayed conspicuous and distinguished morphology (Figure 3) was easily identified even at 40x magnification. The species' valve is cylindrical with rounded ends and formed straight chain which is held by fine marginal processes. Each cell equipped with two chromatophores located at the margin of the valve. The species showed a significant temporal (monthly) variability at $F_{11,35} = 4.118$, $p < 0.01$, with no cell occurrence in July 2009 (Figure 4A). The absence of this species in July 2009 is also concurrent with low total phytoplankton diversity (Figures 4A-4D). The absence of *S. costatum* in July 2009 was hypothesized to closely relate to grazing activity by shrimp larvae. This assumption is further supported by the concurrently low level of total

phytoplankton diversity. Through observation, there was high density of the larvae in the sampling months. However, no investigation was done for further information.

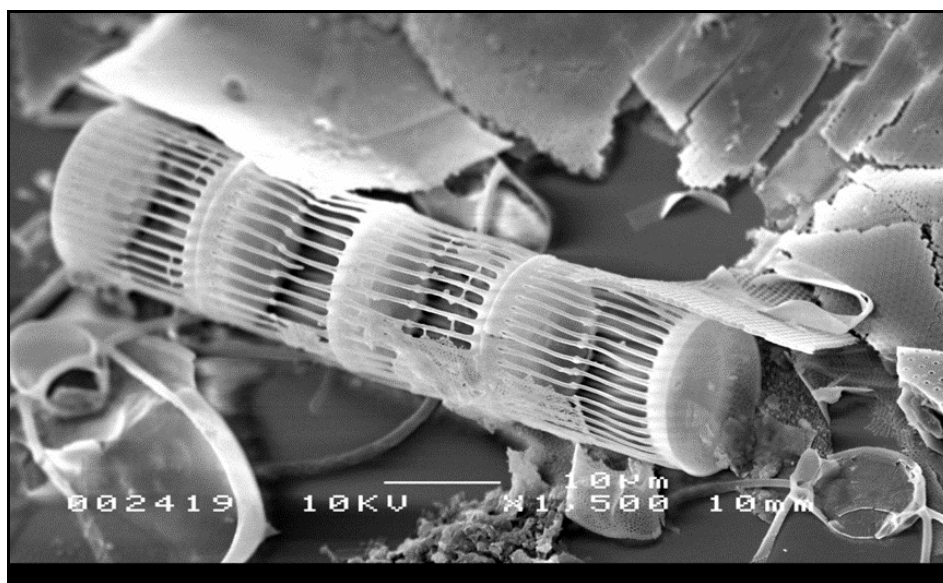


Figure 3. SEM pictograph of the chain forming species, *Skeletonema costatum*.

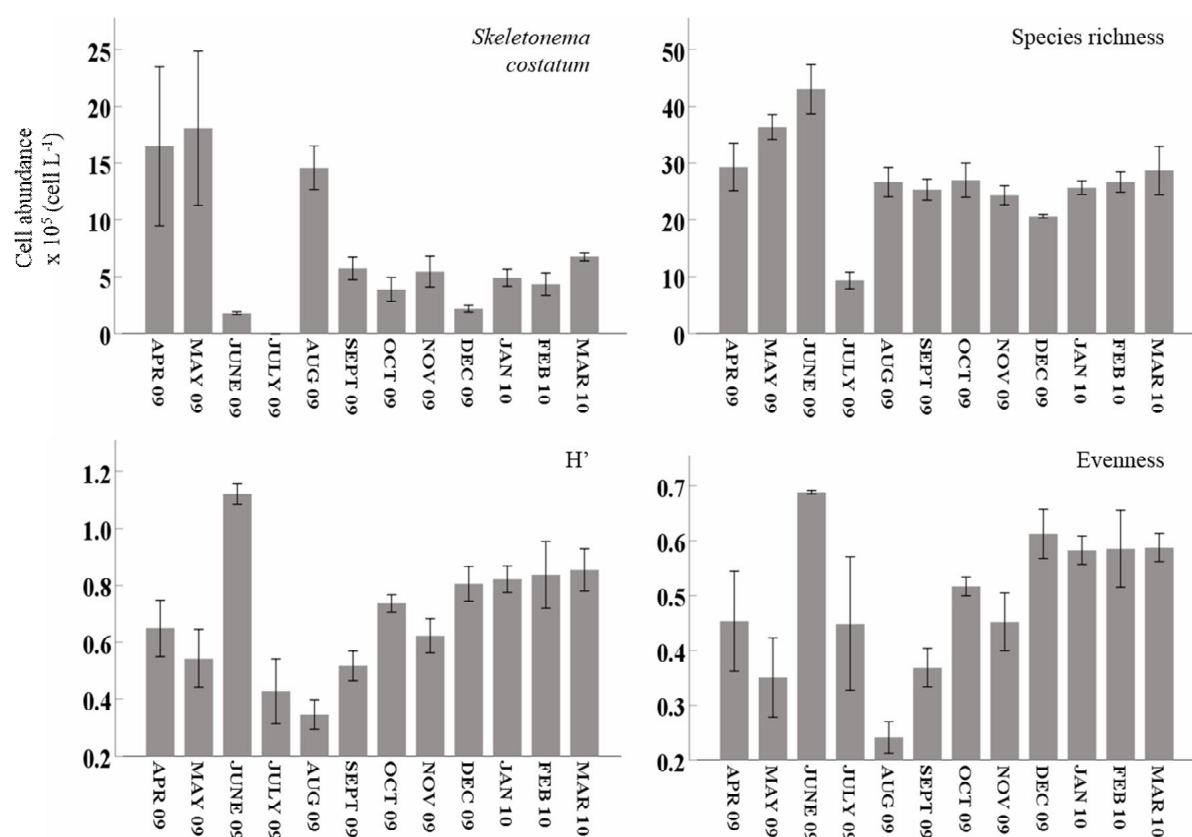


Figure 4. Monthly variability of: A. *S. costatum* cell abundance and total phytoplankton's diversity indices; B. H' ; C. species richness; D. evenness over a year sampling period (n = 9).

Three *S. costatum* bloom events were recorded in three sampling months when the cell abundance was higher than 10×10^5 cells L^{-1} ; in April 2009 ($16.5 \pm 7.0 \times 10^5$ cells L^{-1}), May 2009 ($18.1 \pm 6.8 \times 10^5$ cells L^{-1}) and Aug 2009 (14.6 ± 1.9 cell L^{-1}) (Figure 4A). *S. costatum* cell density in the three months were also significantly higher than other sampling months at $p < 0.001$. Increased in the *S. costatum* cells in the water column showed to have negative effect on the total phytoplankton density, which confirmed by

the high proportion of the species cell density (> 60%) to the total phytoplankton density (Figure 5). The bloom events were also coincided with lower total phytoplankton diversity. Months of April, May and August 09 displayed relatively lower H' and evenness values than most of other months (Figures 4B and 4D, respectively).

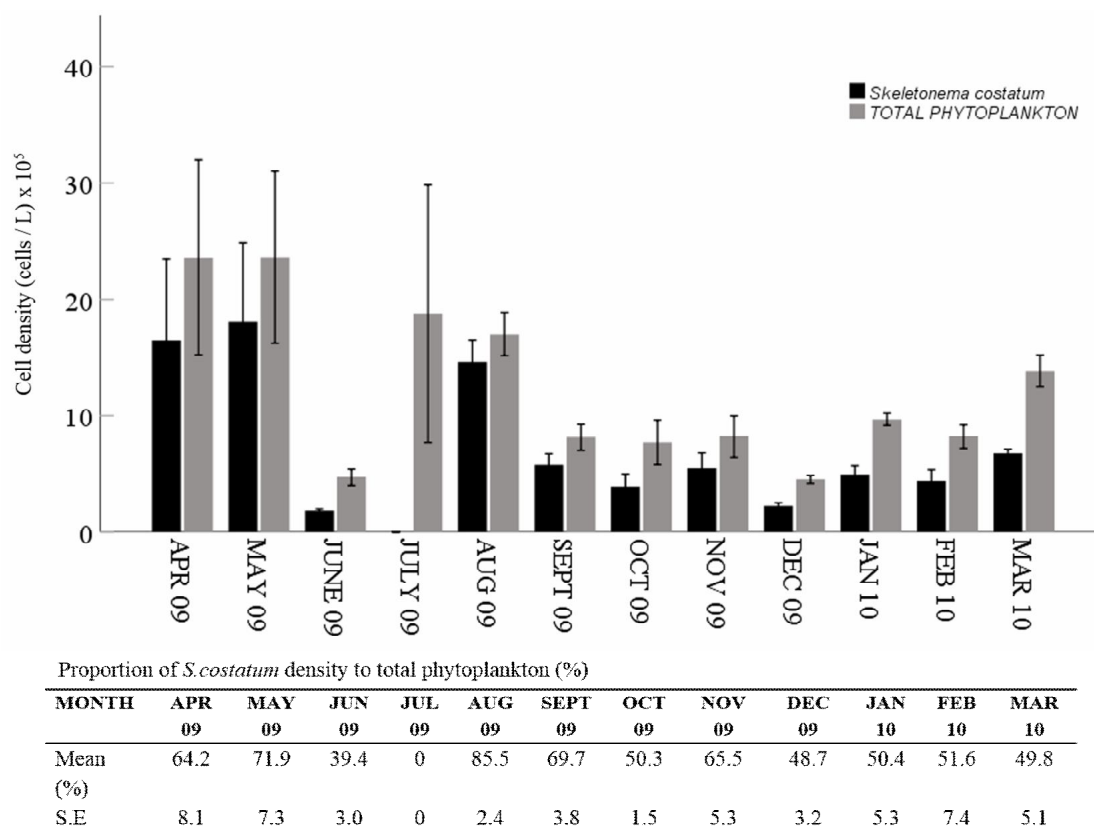


Figure 5. Cell density of *S. costatum* and total phytoplankton. Values are mean±SE. Also details in the figure is the proportion of *S. costatum* cell density to total phytoplankton.

There was a significant negative correlation between the *S. costatum* with both the H' and evenness values of the total phytoplankton (Figures 6A and 6B, respectively). The mentioned negative correlation further supported that the *S. costatum* may be responsible of the decreased of the total phytoplankton diversity at the study site. High *S. costatum* cells abundance at study site showed to cause low total phytoplankton diversity in terms of their equitability (evenness) and not the species richness. The significant ability of high *S. costatum* density (bloom) in decreasing the total phytoplankton's diversity in Malaysia was also reported by Boonyapiwat (1999), who recorded occurrence of only 9.7% relative abundance of other phytoplankton species when *S. costatum* was in high density.

S. costatum cell density at the study site was closely related to the chemical and physical parameters: the nitrate concentration, the temperature and the DO of the water column. Increased in temperature and nitrate significantly positively enhanced the growth of *S. costatum* at $r = 0.471$, $p < 0.05$ (Figure 6C) and $r = 0.716$, $p < 0.001$ (Figure 6D), respectively. Lower DO found to coincide with high *S. costatum* cell density. There was a significant negative correlation between the *S. costatum* cells density with the DO in the water column with the r value of -0.483 , $p < 0.01$ (Figure 6E).

The bloom events of *S. costatum* at the study were potentially initiated when the water temperature and the nitrate concentration were higher than 3 °C (Figure 6C) and 0.7 mg L⁻¹ (Figure 6D) and the DO was lower than 2.6 mg L⁻¹ of water (Figure 6E), respectively. Phosphate, silicate and salinity did not have any significant effect on the occurrence of *S. costatum* cell density at the study site. This present study found that the *S. costatum* species in this ecosystem favoured and became opportunistic in the

temperature ranges between 31.2 to 31.5°C. This temperature range was slightly similar to the range of 31.2-31.3°C that was reported by Huo & Shu (2005) a day before the onset of *S. costatum* bloom in Jiaozhou Bay, China. Unlike other species in genus *Skeletonema*, *S. costatum* proved to survive and successfully grow in wider temperature range of 10-34°C (Kaeriyama et al 2011). This further explained why this unique species is recorded and reported in water bodies in all regions, which makes it a cosmopolitan species. Also, its ability to adapt in such wide temperature ranges must be the reason that supported the species' frequent mono-species bloom in the temperate (Smayda 2004), the Atlantic (Kent et al 1995) and the tropic (Shaik et al 2015).

Nitrate concentration was the only chemical parameter that significantly controlled the occurrence of *S. costatum* at study site. In contrast to offshore study done by Shaik et al (2015), it is the silicate that significantly stimulated the growth of the species. The contra finding could be attributed to the different ecosystem type of the study site, or perhaps, could be related to the study sites' distance to the land. Mangrove ecosystem that lies along coastline and more adjacent to the land, makes it more susceptible to be exposed to nitrate rich anthropogenic runoff. In addition, the ecosystem also receives nitrate input from decomposed detritus from the mangrove vegetation (Roy et al 2012), which potentially intensified the nitrate concentration at the study site. Shallow water of the study site with continuous nitrate availability must be the primary factor for the *S. costatum* to be the dominant phytoplankton species at the study site or generally mangrove ecosystem (Canini et al 2013).

Increased or high water temperature and nitrate concentration at our study site potentially enhanced the growth of *S. costatum* by stimulating the species to form longer and rigid chain. Takabayashi et al (2006) reported that high nitrate-rich and high temperature of more than 17°C initiate an exponential growth in the species, by means of number of cells per chain (up to 8 cells per chain). During high cells abundance of *S. costatum* at the study site, the species was observed to contain of more than 10 cells per chain. Longer chain of *S. costatum* reported to increase buoyancy and consequently increases rate of the cells' photosynthesis by assisting the cells to float and remain at euphotic zone (Takabayashi et al 2006).

Low DO at study site showed to coincide with high *S. costatum* cell density. One reason of the finding could be explained by the fact that *S. costatum* at study site was more efficiently utilizing the low DO than other phytoplankton species, therefore, outgrew them. Although technically high biomass due to the bloom event must produce high DO (Huo & Shu 2005), it could also lead to hypoxia condition ($< 2.8 \text{ mg DO L}^{-1}$ water) (Smayda 2004). Marraro et al (2016) reported that the phytoplankton species that caused bloom normally is consumed by zooplankton or dies. Therefore, the next potential reason could be that the dead phytoplankton and the faecal deposit from the zooplankton are decomposed by the bacteria which require oxygen in the water column. Consequently, low DO is created when the oxygen needs by bacteria to continue decomposing the mentioned materials is higher than the oxygen produces by the phytoplankton biomass. Huo & Shu (2005) also observed a low DO during *S. costatum* bloom in Jiaozhou Bay when the bloom is collapsing.

High cells density of *S. costatum* during high nitrate ($> 0.6 \text{ mg L}^{-1}$) indicates that nitrate concentration of more than 0.4 mg L^{-1} (Rahaman et al 2013) at the study site was an extreme level that only certain species could tolerate (Titman 1976). *S. costatum* that reported as a good competitor in not only nitrate but also phosphate replete conditions (Hu et al 2011) must opportunistically utilize this condition to double up their growth by increasing the length of its chain (discussed in previous paragraph).

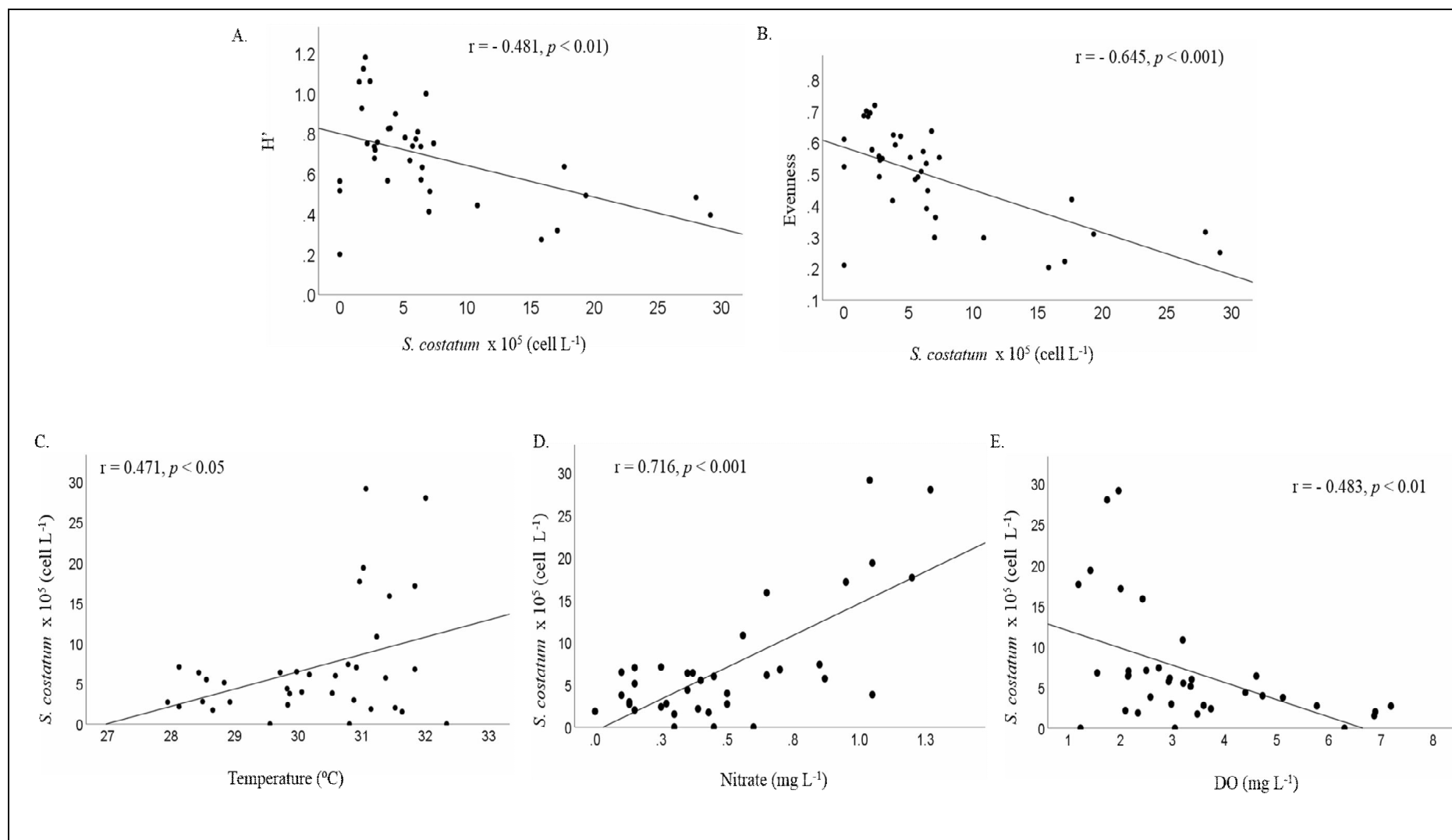


Figure 6. Significant negative relationship between the cells abundance of *S. costatum* with; diversity indices the; A. H' and B. evenness and significant relationship between the cells abundance of *S. costatum* with; C. temperature ($n = 36$), D. nitrate ($n = 36$) and E. DO ($n = 36$). r is the strength of the Pearson correlation coefficients between two mentioned variables.

Conclusions. *S. costatum* was a dominant phytoplankton species at the study site, which is a mangrove ecosystem. This study shows increased nutrients primarily nitrate which is believed to have originated from inland and aquaculture activities stimulated the growth of this chain and bloom-forming *S. costatum*. In addition, increased temperature also showed to have significant positive effect on the species cells abundance. Because nitrogen had great effect on the *S. costatum*, it is perhaps worth to also include the effect of other forms of nitrogen such as the ammonium and urea in the future study. Although we found the negative relation between the *S. costatum* and the DO. It is still unsure whether it is the low DO level that caused the bloom or the bloom has caused the low DO. Monitoring programme of total phytoplankton and bloom events in mangrove ecosystem is an important approach of sustainable management of the mangrove ecosystem not only in Malaysia, but also in other regions.

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Authors:

Nurul Shahida Redzuan, School of Marine and Environmental Science, University Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia, e-mail: nurulshahida@umt.edu.my

Pozi Milow, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia, e-mail: pozimilow@um.edu.my

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