

Monthly Chaetocerotaes diversity and abundance, and its relationship with water physicochemical parameters and phytoplankton diversity in Carey Island mangrove ecosystem, Malaysia

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Abstract. Redzuan NS, Milow P. 2021. Monthly Chaetocerotaes diversity and abundance, and its relationship with water physicochemical parameters and phytoplankton diversity in Carey Island mangrove ecosystem, Malaysia. *Biodiversitas* 22: 2919-2927. Globally, phytoplankton of order Chaetocerotaes has been reported to cause monospecific bloom events. The blooms that normally lead to ecosystem instability, also physically responsible for histological and physical damages to fishes. Regarding that order Chaetocerotaes often display significant temporal variability, this study aimed to investigate the monthly variability of species composition and cell density of phytoplankton of order Chaetocerotaes during high tide period immersion were investigated over a year period. In addition, this present study also aimed to analyze the relationship between Chaetocerotaes diversity and abundance with physical and chemical parameters that potentially controlling their temporal variability. Samplings were carried out from April 2009 until March 2010 in the mangrove ecosystem of Carey Island, Selangor, Malaysia. Of 84 phytoplankton taxa recorded, 18 species belonged to order Chaetocerotaes, i.e. the genera *Bacteriastrum* and *Chaetoceros*. Although initially the sampling was designed to study the overall phytoplankton, the study also aimed at high temporal variability in Chaetocerotaes cells density has called for this paper to be written. Interestingly, order Chaetocerotaes showed to have negative correlation with the total phytoplankton species. Chaetocerotaes blooms at the study site were represented by multispecies blooms, with *Chaetoceros curvisetus* as the highest species in the events. Dissolved oxygen, on the other hand, showed to have negative correlation Chaetocerotaes' cells density. All of the species, except the *Chaetoceros subtilis* and *Chaetoceros neglectus* displayed significant temporal monthly variability across twelve months of sampling. Nitrate, phosphate and temperature are potentially the parameters that stimulate the growth of the order, consequently causing bloom events.

Keywords: Chaetocerotaes, Carey Island, mangrove ecosystem, *Chaetoceros*, *Bacteriastrum*

INTRODUCTION

Chaetocerotaes is one of the common orders within the division of Bacillariophyta that inhabits both marine and mangrove ecosystems in the tropical region. Attention has been given to this phytoplankton order due to its progressively increasing importance in aquaculture, medicine and biotechnology. Chaetocerotaes have been documented as vital source of antifreeze protein (Gwak et al. 2010) and source of food in aquaculture feeds (Hashimoto et al. 2008; Kang et al. 2009). Ecologically, Chaetocerotaes are frequently reported as the phytoplankton group that responsible for annual early spring bloom by forming the *Chaetoceros*-dominated chain in various regions of the world (Ishikawa and Furuya 2004; Booth et al. 2002; Poulsen and Reuss 2002).

The member of the order Chaetocerotaes can be distinguished from the other phytoplankton groups by means of their long spiny setae that protruding from their valves (Hasle and Syvertsen, 1997). This unique structure also reported being responsible to cause fish kills and harmful to various types of invertebrates all around the world (Ochieng and Mary 2013; Bosch-Belmar et al. 2017;

Paterson et al. 2017). Bruno et al. (1989) reported that fish mortality is commonly attributed to this phytoplankton order. Their silicified frustules and setae are able to directly clog and penetrate into fishes' gills, causing severe histological damage.

Temporal distribution of Chaetocerotaes often demonstrates high variability due to physicochemical factors (Brunet and Luzon 2003; Carter, et al. 2005; Facca and Sfriso 2007; Frankovich et al. 2010). In Mediterranean coasts, temperature, salinity and phosphate concentrations in the water column were proven to stimulate dominant Chaetocerotaes species succession (Bosak et al. 2016). The study also stated that warmer seasons such as in summer, frequently triggered monospecific bloom events. In Malaysia, Chaetocerotaes primarily from the genus of *Chaetoceros* were commonly reported as dominant coastal phytoplankton species (Razali et al. 2015; Chang Lim et al. 2014; Sidik et al. 2008). Chaetocerotaes species in the aquaculture area of Aman Island, Malaysia, positively correlate to physical parameters, pH, salinity, and temperature (Razali et al. 2015). Although there is yet event of severe fish kills and bloom by order Chaetocerotaes in Malaysia, increasing anthropogenic

emissions, active aquaculture and climate change, possibly will increase the potential of bloom events to happen. Moore et al. (2008) hypothesized that global warming potentially initiates high water body temperature, more acidic aquatic and marine pH and also changes in evaporation patterns. Although the mentioned changes are most likely to lead to higher frequency of harmful algal bloom events, very little attention has been given to study the ecology of these harmful blooms.

To date, there is still no comprehensive study has ever been done exclusively on the Chaetocerotaes ecology in Malaysia. Most attention has only been given to investigate the distribution and occurrence of total phytoplankton, not specifically on only one order or group. Therefore, in this study, phytoplankton was collected to investigate and update the species composition of Chaetocerotaes variation over a year. Therefore, this study aimed to investigate the monthly variability of species composition and cell density of Chaetocerotaes phytoplankton over a year in a mangrove ecosystem of Carey Island, Selangor, Malaysia. As part of monitoring program, this temporal distribution study also includes the relationship between Chaetocerotaes with physicochemical parameters that potentially controlling their variability in the water column. Considering the anticipated environmental changes caused by anthropogenic and climatic factors in the future, we expected this study to provide an informative record on the dynamic of this phytoplankton group, which is also listed as the group that has high potential cause global harmful blooms. Mangrove ecosystem was chosen as the study site in this scientific investigation to better understand the ecology of estuarine Chaetocerotaes. The ecosystem that is

considered as an extreme habitat for the phytoplankton, should provide a new underpinning ecological information on this phytoplankton group, the Chaetocerotaes.

MATERIALS AND METHODS

Study site

Mangrove ecosystem (02°92'N, 101°35'E) of Carey Island, Malaysia was chosen as the study site (Figure 1). The ecosystem lies along Langat River estuarine Banting, Selangor state, Malaysia. Listed by the Malaysian Wetland Working Group as mangrove island, Carey Island is mostly surrounded by mangrove areas, thus, making the ecosystem a suitable area to study phytoplankton of the genus Chaetocerotaes that inhabit the mangrove. Influx of water from Sungai Langat that flows into the mangrove ecosystem makes the water brackish and slightly acidic. Langat River flows along 120 km westward into Straits of Malacca and receives huge amounts of domestic and industrial wastes each year which normally will also affect the chemical and physical profiles of the research area.

Three stations were chosen to obtain the phytoplankton samples, namely ST1, ST2, and ST3 (Figure 1). The stations or sites were chosen based on their horizontal distance to the river mouth that displayed significant salinity gradient. ST1 was located upstream of the river and was adjacent to a shrimp aquaculture pond. ST2 positioned in a radius of 50 m to an oil palm process plant, while ST3 was the nearest site to the river mouth.

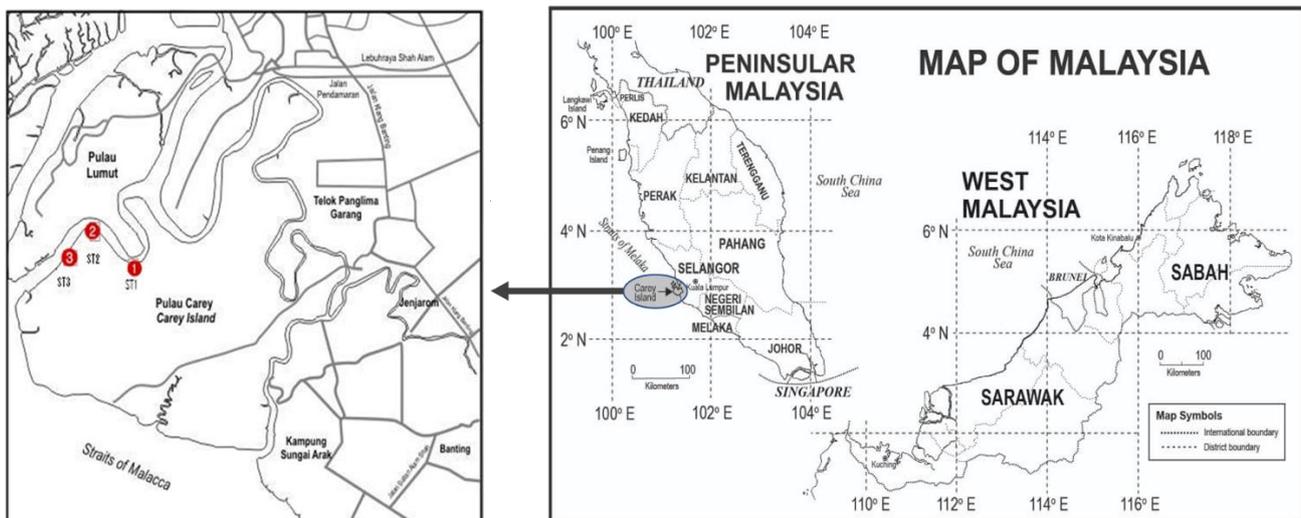


Figure 1. Map of Pulau Carey, Selangor, Malaysia and the study sites which consisted of three observation stations (i.e ST1, ST2 and ST3)

Phytoplankton sampling, enumeration and identification

Monthly phytoplankton samplings were carried out from April 2009 until March 2010. The samplings were conducted once (for 1 day) in a month, with temporal gap between sampling occasions of 28-30 days. Triplicate samples were obtained using a 30 µm wire mesh plankton net and were preserved in 4 % formalin for identification and enumeration purposes. A flow meter was attached at the mouth of the plankton net to determine the volume of water that passed through it. One ml of preserved algae sample was pipetted into utermöhl sedimentation tube, in which one drop of Lugol's iodine was added. Slides were prepared following the Bellinger and Sigeo (2010) procedure. Phytoplankton cells were enumerated by dividing the sedimentation tube into four divisions. The enumeration was carried out from one division to another (Evans 1972) and was expressed in cells L⁻¹. The slides prepared were also used in identification process of both the phytoplankton. Phytoplankton identification was done based on their morphology, down to genus or species level. Taxonomic keys by Tajudin and Salleh (2006) and Hasle and Syvertsen (1997) were used and referred for identification process.

Water sampling and its physicochemical measurement

Water samples for chemical analyses that were collected using Ruttner sampler were transferred into polythene bottles (500 ml) and preserved using four percent (%) formalin. Measurement of physical factors such as water temperature, pH, salinity, water conductivity (CND), dissolved oxygen (DO) and total dissolved solid (TDS) were recorded in situ using a handheld YSI Model 556 MPS. Whereas, chemical analyses such as phosphate (PO₄³⁻), nitrate (NO₃²⁻), silicate (SiO₃) and sulfate (SO₄²⁻) were carried out in the laboratory. The collected water samples for the water chemical analyses were filtered using Whatman-microfilter paper. All of the nutrients analyses were spectrophotometrically measured using Spectrophotometer DR4000 HACH at the respective wavelength for each of the chemicals. The chemical concentrations were expressed in mg L⁻¹.

Statistical analyses

Monthly total phytoplankton and Chaetocerotaes species diversity were investigated using evenness (*E*) and Shannon Diversity Index (*H'*). The indices were calculated using the Multi-Variate Statistical Package (MVSP) 3.1 software (Kovach, 1999). All of the data obtained from the three stations were pooled because of their dependency (n=9). One-way Analysis of Variance (ANOVA) was performed on the data of Chaetocerotaes cells density and physicochemical parameters to determine the monthly variability for twelve months. The *F* values that contributed to different temporal variations at *p* < 0.05 were considered significant. Principal Component Analyses (PCA) was performed using FactoMineR in R Statistical Software. The analyses were done on the log-transformed (n+1) data of Chaetocerotaes' cells density and the measured physicochemical parameters. The individual scores of the

PCA were then analyzed for temporal monthly variability of Chaetocerotaes species composition. Only the first and the second component of the PCA were retained and used in the results and discussion. Also, the ordination of each of Chaetocerotaes species and Physico-chemical parameters in dimension 1 (Dim. 1) and dimension 2 (Dim. 2) of the PCA were used to investigate any possible correlation between each of the species with the measured parameters.

RESULTS AND DISCUSSION

Temporal physicochemical variability

Nitrate and phosphate concentrations in the water column at the study sites significantly varied across the months over the year at $F_{11,35}=16.48$, $p < 0.001$ and $F_{11,35}=3.491$, $p < 0.05$, respectively. While both silicate and sulfate concentrations showed non-significant monthly variation. DO with annual mean value of 3.34 ± 0.21 mg L⁻¹ was the only physical parameter that insignificantly varied over the year (Table 1), whereas all the other parameters were temporally varied at monthly scale at $p < 0.001$ (Table 1). Details data on the monthly mean of the physicochemical parameters are presented in Table S1 in supplementary data.

Temporal variability of Order Chaetocerotaes

A total of 84 species of phytoplankton ((Redzuan and Milow 2019) were recorded at study site across the twelve consecutive months of sampling. Out of the 84 species, 18 species were from order Chaetocerotaes, which comprised of two genera, *Bacteriastrium* and *Chetoceros* (Figure 2). Both were two common genera recorded in Carey Island mangrove ecosystem. This finding is in agreement with a study done by Bhattacharjee et al. (2013) that reported the genus *Chaetoceros* as one of dominant species in Sundarbans mangrove, India. The genus *Bacteriastrium* was absent from the Sundarbans mangrove ecosystem, but was reported in the estuary ecosystem of the Sundarbans (Manna et al. 2010). Both genera of Chaetocerotaes are commonly reported in Malaysia coastline (Chang Lim et al. 2014; Sidik et al. 2008).

Table 1. The results of physical and chemical parameters (n=12) measured at the study site (Mean value ± SE)

Physical and chemical parameters	
Chemical parameters	
Nitrate (NO ₃ ²⁻)	0.50 ± 0.06 mg L ⁻¹
Silicate (SiO ₃)	2.53 ± 0.25 mg L ⁻¹
Phosphate (PO ₄ ³⁻)	0.94 ± 0.1 mg L ⁻¹
Sulfate (SO ₄ ²⁻)	55.44 ± 3.45 mg L ⁻¹
Physical parameter	
Water temperature (°C)	30.26 ± 0.21
Conductivity (µs/cm)	42230.72 ± 533.96
Dissolved oxygen (mg L ⁻¹)	3.34 ± 0.27
Total dissolved solid (mg L ⁻¹)	26.04 ± 0.27
pH	7.44 ± 0.07
Salinity (ppt)	24.84 ± 0.45

Species from both genera contributed to 21.4 % of the total phytoplankton species. Species composition of order Chaetocerotales occurred in seven months (April 2009, May 2009, June 2009, August 2009, January 2010, February 2010, and March 2010) out of the twelve sampling months. Although the species were absent in five out of the twelve sampling months, their occurrence showed to have significant impact on the diversity of the total phytoplankton. Increased in the cells abundance of Chaetocerotales showed to have negative effect on the total phytoplankton diversity (Table 2). This phenomenon could potentially remark the onset of bloom by order Chaetocerotales at the study site.

The highest cell density was recorded in April 2009 (Figure 2) (Table 2) with recorded total cells abundance of 360×10^3 cells L^{-1} . The month was also reflected by comparably high H' and E values of 3.194 and 0.94 (Table 2), respectively, with *Bacteriastrium comosum* as the highest contributor of the order's cells abundance (131.3×10^3 cells/L) (Figure 2). However, this finding coincided with relatively low H' and E values of the total

phytoplankton (Table 2). Both values were 1.607 and 0.316, respectively. Cells abundance of the *Chaetoceros curvisetus* was significantly higher than other Chaetocerotales species in the months which the species occurred (Figure 2). The species' highest cells abundance was in May 2009 ($149 \times 10^3 \pm 9207.49$ cells/L) and concurrent with the relatively low H' and E values of both total phytoplankton (Table 2) and order Chaetocerotales (Table 2). Each of the order Chaetocerotales species showed a significant monthly variability (all significant at $p < 0.05$) with exception of two *Chaetoceros* species, the *C. neglectus* and *C. subtilis*. High H' and E among the Chaetocerotales group at the study site indicated higher chance of multispecies bloom rather than monospecific bloom events happening. This high multispecies' cells abundance of Chaetocerotales at study site is likely to mimic the multispecies bloom of the genus *Chaetoceros* on the Mediterranean coast during autumn which frequently triggered by mixing events of the water column (Bosak et al. 2016).

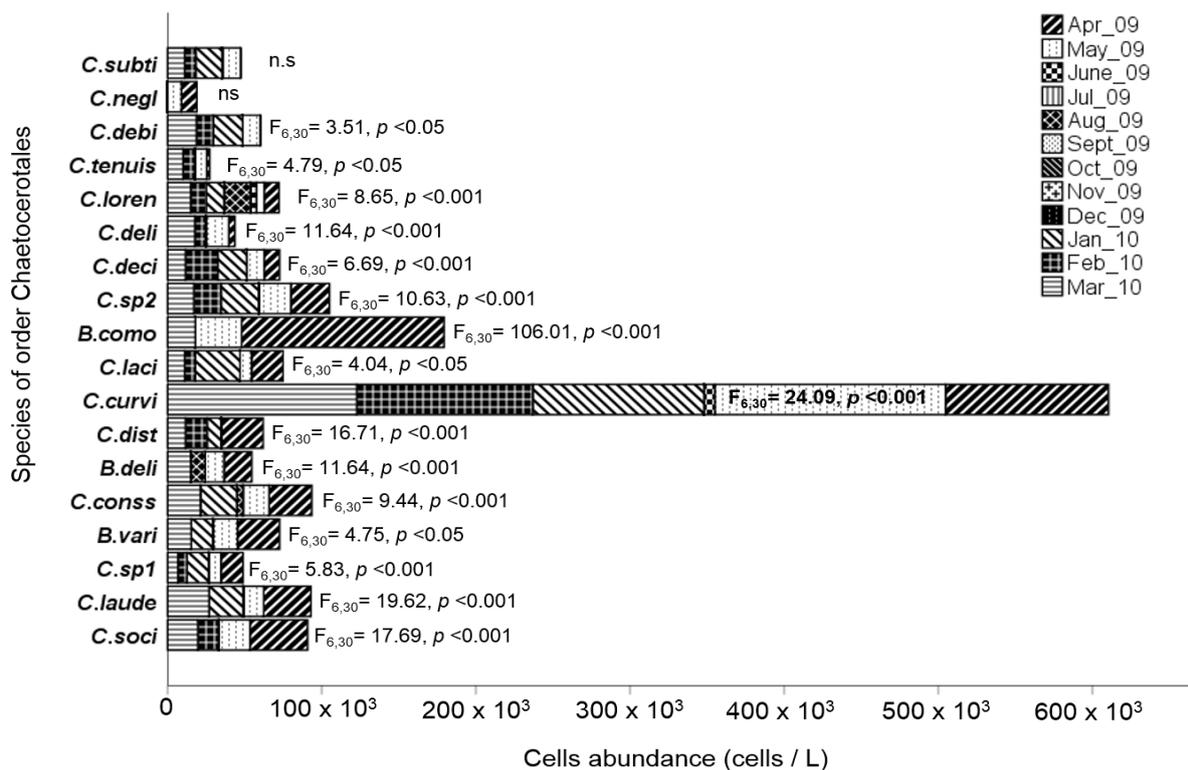


Figure 2. Cells density of order Chaetocerotales species composition across the twelve sampling months. Notes that standard error (SE) values are not presented in the figure. Also included the F-value and significant level for each of the species' monthly ANOVA output. Note: *C.subti* = *C. subtilis*; *C.negl* = *C. neglectus*, *C.debi* = *C. debilis*, *C. Tenuis* = *C. tenuissimus*, *C.loren* = *C. lorenzianus*, *C.deli* = *C. delicatulum*, *C.deci* = *C. decipiens*, *B.como* = *B. comosum*, *C.laci* = *C. lacinosum*, *C.curvi* = *C. curvisetus*, *C.dist* = *C. distans*, *B.deli* = *B. delicatulum*, *C.cons* = *C. constrictus*, *B.vari* = *B. varians*, *C.laude* = *C. lauderii* and *C.soci* = *C. socialis*.

Table 2. Cells density (cells L⁻¹), species richness (number of species), diversity (Shannon Weiner Index) and evenness (*E*) of total phytoplankton and order Chaetocerotales at Carey Island across the sampling months

Sampling month	Apr 09	May 09	Jun 09	Jul 09	Aug 09	Sep 09	Oct 09	Nov 09	Dec 09	Jan 10	Feb 10	Mar 10
Total phytoplankton												
Cells density x 10 ³ (cells L ⁻¹)	3630	3540	579	4033	1969	912	627	929	515	1065	869	1257
No. of species	34	38	50	7	27	24	24	26	21	24	24	23
Shannon Weiner Index (log ₂)	1.607	1.315	3.924	1.716	1.059	1.373	2.52	1.707	2.448	2.448	2.218	2.503
Evenness (<i>E</i>)	0.316	0.251	0.695	0.611	0.223	0.299	0.55	0.363	0.557	0.534	0.484	0.553
Order Chaetocerotales												
Cells density x 10 ³ (cells L ⁻¹)	360	320	0	0	66	0	0	0	0	267	191	291
No. of species	11	14	0	0	5	0	0	0	0	8	7	11
Shannon Weiner Index (log ₂)	3.195	2.86	0	0	2.114	0	0	0	0	2.359	1.893	3.236
Evenness (<i>E</i>)	0.924	0.751	0	0	0.91	0	0	0	0	0.786	0.674	0.936

Potential relationship between Chaetocerotales and physical and chemical parameters

A Principal Component Analysis (PCA) was performed on the log (n+1) of order Chaetocerotales cells abundance and the measured physical and chemical parameters. Initially, four and six chemical and physical parameters, respectively, were chosen by the PCA. However, only two chemicals (nitrate and phosphate) and four physical parameters (DO, temperature, pH and TDS) were retained in the PCA together with the Chaetocerotales species.

The dimension 1 (Dim. 1) and 2 (Dim. 2) of the PCA explained a total of 61.1 % of the total variation. The Dim. 1 which explained 41.3 % of the total variation (Figure 3A) displayed the relationship between the seventeen Chaetocerotales species with nitrate, phosphate, temperature and DO. Variable factor map of the PCA showed that ten of the Chaetocerotales species and nitrate concentration in the water column were strongly and positively correlated with the positive axis of the Dim. 1. Species of *C. socialis*, *C. lauderii*, *C.sp1*, *C.constrictus*, *B. delicatulum*, *C.distans*, *C. curvisetus*, *C.laciniosum*, *B. comosum*, *B. varians*, and *C.sp2* were the species together with nitrate that displayed significantly positive correlation with the Dim. 1 ($r > 0.7$) of the PCA. The mentioned species occurred in relatively high abundance in the three sampling months that recorded with high nitrate concentration (> 0.5 mg L⁻¹) (April 09; 1.2 ± 0.1 mg L⁻¹; May 09; 1.0 ± 0.1 mg L⁻¹; and; Mar 2010; 0.7 ± 0.1 mg L⁻¹) (Figure 3A and 3B). This further supported that nitrate concentration potentially significantly and strongly enhanced some Chaetocerotales species in the water column of the study site.

Nitrate in the water column was found to have strong effect on the Chaetocerotales species with major cells density, which were the *B. comosum* and *C. curvisetus*, and a few other species. The significant relationship between the Chaetocerotales in Carey Island could be attributed to their adaptation to living in the extreme mangrove

ecosystem. Other than nutrients from anthropogenic runoff, mangrove also exposes to nutrient enrichment from detrital-sourced nitrogen (Roy et al. 2012), which possibly enhanced the nitrate concentration in this ecosystem. The positive relationship between nitrate and Chaetocerotales was possibly one of exclusive ecology of Chaetocerotales inhabiting the mangrove. This phytoplankton group ability to opportunistically use up the nitrate in this extreme condition may be the answer to their high cells abundance in high nitrate concentration in the months of April 2009, May 2009, and March 2010.

Phosphate concentration and the temperature of the water column were the other parameters that were retained in the PCA. The parameters and six species of Chaetocerotales namely the *C. decipiens*, *C. delicatulum*, *C. tenuissimus*, *C. debilis*, *C. lorenzianus*, and *C. neglectus* showed a positive correlation with the Dim. 1 at $r < 0.7$. Nitrate, phosphate and temperature were potentially the important parameters that positively stimulating the growth of order Chaetocerotales at the study site.

Positive relationship between the genus *Chaetoceros* and *Bacteriastrum* with phosphate concentration in the water column in this present study, has also been reported in numerous phytoplankton studies (Bosak et al. 2016; Yamaguchi et al. 2005a, b). This order of phytoplankton has proven to utilize a wide range of phosphate sources. They can take up and utilize the common source of organic phosphate and source of phosphate from inorganic phosphate and monophosphate monoester compound (Yamaguchi et al. 2005a) and from phosphate diester (Yamaguchi et al. 2005b). This ability may enable Chaetocerotales to outstand other phytoplankton species in response to repletion or depletion of this source of nutrient. The affinity of order Chaetocerotales towards wide range of phosphate possibly further explained their high cells abundance or bloom event in aquaculture, coastline and mangrove ecosystem.

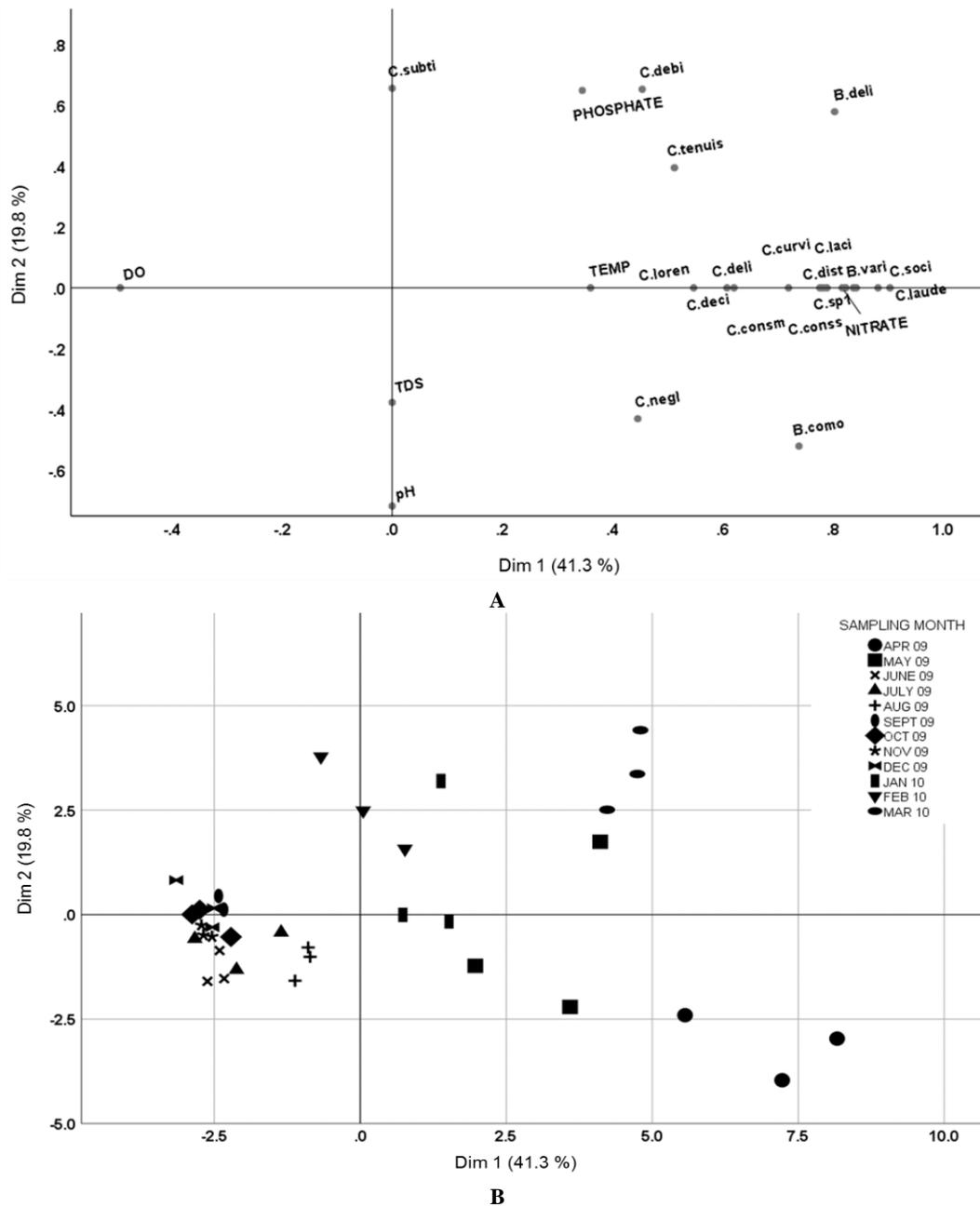


Figure 3. The output of Principal Component Analysis (PCA) displaying the relationship between Chaetocerotales and the physical and chemical parameters. A. Variables factor map and; B. Individual factor map of the PCA that was performed on the Chaetocerotales species composition and physical and chemical data (Table S1)

The low DO in the water column was possibly closely related to high Chaetocerotales cells density at the study site. Hypothetically, although high Chaetocerotales biomass due to their high density may lead to high DO due to high photosynthesis rate (Begum et al. 2015; Huo and Shu 2005), it could also cause hypoxia condition (Smayda 2006). Towards the end of high density or Chaetocerotales bloom event, most of the cells will be consumed by zooplankton or naturally dead (National Science and Technology Council 2016). Fecal matter from zooplankton and dead cells will be decomposed by bacteria which demands oxygen higher than oxygen being produced by the

Chaetocerotales species. Huo and Shu (2005) also reported low DO in the water column when period of high species density (bloom) *Skeletonema costatum* ended in Jiazhou bay, China. The next hypothesis is simply because the species in the Chaetocerotales opportunistically utilized the low DO concentration per L of water at the study site. Finding by Ochieng and Mary (2013) further supported this hypothesis by stating that it is the oxygen depletion towards the end of phytoplankton bloom that causes fish kills along the Kenyan Coasts. Also, DO level in the water column was higher in the months in which order Chaetocerotales was absent or in low density.

In contrast to Dim. 1, only six species of order Chaetocerotaes were significantly correlated with Dim. 2. The Dim. 2 however, only explained 19.8 % of the total variation and displayed the relationship between some species of Chaetocerotaes with TDS and pH. Species *B. delicatulum*, *C. tenuissimus*, *C. debilis* and *C. subtilis* were the species that positively correlated with the Dim. 2 of the PCA. Negative correlation between TDS and pH with the Dim. 2 potentially supported the negative relationship between the mentioned species with these parameters. The presence of *B. comosum* and *C. neglectus* were only recorded in the month of Apr and May 2009. Both months were characterized by comparably high TDS concentration and pH in the water column (Figure 3B). Four parameters, i.e., the silicate, sulfate, CND, and salinity level in the water column did not have any significant correlation with both Dim. 1 and Dim. 2 of the PCA.

The species of order Chaetocerotaes did not show any significant relationship with silicate concentration in the water column of Carey Island. Silicate concentration that showed a non-significant temporal variability throughout the sampling period could be the reason for this finding. The non-significant temporal variability displayed by silicate suggested that this nutrient potentially was likely available at sufficient concentration throughout the sampling period. Therefore, was not the limiting factor for Chaetocerotaes growth at the study site.

The absence of order Chaetocerotaes in few of the sampling months was hypothesized to closely relate to grazing activity by shrimp larvae. Through observation, there was high density of the larvae in the sampling months in which Chaetocerotaes were not recorded. However, no investigation was done for further information.

In conclusion, with regards to their high cells abundance that relatively and negatively correlated with the total phytoplankton diversity, it could be concluded that Chaetocerotaes is one group of phytoplankton that has significant ecological impact on mangrove ecosystem. Their positive response towards increased nitrate and phosphate is another matter that needs further attention. Therefore, it is perhaps worth including other forms of nitrogen and phosphate in future studies. In addition, their high density occurrence that negatively correlated with DO level also potentially able to cause hypoxia condition in the water column may lead to ecosystem instability. More works are needed, and more hypotheses need to be tested to fully understand the ecology of order Chaetocerotaes inhabiting mangrove ecosystems.

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Table S1. Monthly variability of physical and chemical parameters at the study site

Parameter		Apr 09	May 09	Jun 09	Jul 09	Aug 09	Sep 09	Oct 09	Nov 09	Dec 09	Jan 10	Feb 10	Mar 10
Nitrate (mg L ⁻¹)	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	1.17	0.99	0.15	0.45	0.72	0.21	0.36	0.21	0.32	0.4	0.27	0.73
	Std. Error	0.07	0.06	0.09	0.09	0.12	0.08	0.12	0.05	0.09	0.05	0.07	0.06
Phosphate (mg L ⁻¹)	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	0.83	0.79	0.47	0.82	0.79	1.5	0.48	0.49	0.66	0.98	1.52	1.97
	Std. Error	0.13	0.30	0.09	0.44	0.28	0.44	0.11	0.05	0.18	0.11	0.26	0.28
Silicate (mg L ⁻¹)	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	2.89	3.22	1.49	4.32	3.5	2.04	2.52	1.98	0.93	2.66	2.16	2.62
	Std. Error	0.55	0.41	0.23	2.28	0.93	0.52	0.80	0.36	0.22	0.53	0.40	0.39
Sulfate (mg L ⁻¹)	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	54.5	89.83	69.5	55.17	43.5	50.33	57.67	66.67	52.5	35.17	52.33	38.17
	Std. Error	1.61	15.80	10.69	9.43	8.55	12.60	19.16	12.20	3.28	8.74	3.09	2.77
Temperature (°C)	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	31.17	31.16	31.44	30.9	31.51	30.17	30.13	28.87	28.25	29.44	29.08	30.94
	Std. Error	0.44	0.11	0.15	0.80	0.18	0.38	0.61	0.57	0.21	0.51	0.39	0.49
CND	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	41457.7	44528.3	44788	43318.3	43510.7	37929.3	37964.3	37586.3	43257.7	45754.7	42650.3	44023
	Std. Error	438.89	79.95	195.64	1131.17	526.27	3307.35	2028.74	1104.91	534.50	840.88	318.63	304.26
TDS (mg L ⁻¹)	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	26.57	27.4	29.17	25.96	25.38	25.94	24.6	24.05	26.48	27.78	24.92	24.19
	Std. Error	0.31	0.04	0.12	0.77	0.28	0.02	0.21	0.13	0.09	0.33	0.79	0.41
Salinity	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	25.85	28.62	28.77	25.29	24.63	20.6	21.33	22.66	25.97	25.24	24.52	24.6
	Std. Error	0.51	0.06	0.14	0.75	0.26	0.25	1.24	0.60	0.10	1.87	1.09	0.85
DO (mg L ⁻¹)	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	1.84	2.11	5.37	3.53	2.55	3.96	4.04	2.75	4.26	3.76	3.43	2.42
	Std. Error	0.40	0.44	1.51	1.48	0.35	0.91	0.87	0.44	1.52	0.81	0.16	0.43
pH	N	3	3	3	3	3	3	3	3	3	3	3	3
	Mean	7.92	7.86	7.64	7.47	7.86	7.46	7.2	7.82	6.9	7.27	6.92	6.89
	Std. Error	0.07	0.02	0.19	0.20	0.01	0.16	0.17	0.02	0.15	0.31	0.07	0.08