

Effects of eutrophication on diatom abundance, biovolume and diversity in tropical coastal waters

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Received: 19 December 2016 / Accepted: 27 July 2017 / Published online: 3 August 2017
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Abstract Diatom abundance, biovolume and diversity were measured over a 2-year period along the Straits of Malacca at two stations with upper (Klang) and lower (Port Dickson) states of eutrophication. Diatom abundance, which ranged from 0.2×10^4 to 21.7×10^4 cells L^{-1} at Klang and 0.9×10^3 – 41.3×10^3 cells L^{-1} at Port Dickson, was influenced partly by nutrient concentrations. At Klang, the diatoms were generally smaller and less diverse ($H' = 0.77 \pm 0.48$) and predominated by *Skeletonema* spp. ($60 \pm 32\%$ of total diatom biomass). In contrast, diatoms were larger and more diverse ($H' = 1.40 \pm 0.67$) at Port Dickson. *Chaetoceros* spp. were the most abundant diatoms at Port Dickson but attributed only $48 \pm 30\%$ of total diatom biomass. Comparison of both Klang and Port Dickson showed that their diatom community structure differed and that eutrophication reduced diatom diversity at Klang. We also observed

how $Si(OH)_4$ affected the abundance of *Skeletonema* spp. which in turn influenced the temporal variation of diatom community at Klang. Our results highlighted how eutrophication affects diatom diversity and community structure.

Keywords Diatom abundance · Diatom community · Diatom biovolume · Tropical waters

Introduction

Diatoms are unicellular phytoplankton in the class Bacillariophyceae and are characterized by a silica outer shell or frustule (Round et al. 1990). They play an important role in the ocean food web by supplying carbon and energy to the pelagic ecosystem, and they account for about 20% of the global primary production (Field et al. 1998). Due to their tight coupling with carbon cycle and their rapid response to environmental changes, abundance, biovolume and diversity of diatoms are vital indicators for understanding how marine ecosystems respond to eutrophication. These indicators have been used to monitor environmental conditions and water quality (Lee 2003; Rott et al. 2003; Gameiro et al. 2007; Potapova and Charles 2007).

Diatoms are widely distributed (Malviya et al. 2016) and are particularly common in nutrient-rich regions and coastal waters (Smetacek 2012). However, reports of diatom distribution are lacking in the Strait of Malacca (OBIS 2016), even though the Strait of Malacca is an important source of renewable and non-renewable

Electronic supplementary material The online version of this article (doi:10.1007/s10661-017-6147-4) contains supplementary material, which is available to authorized users.

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resources and is also a key international shipping route. It is therefore vital to assess diatom abundance, biovolume and diversity in the Strait of Malacca as it is experiencing significant eutrophication due to rapid economic development and an increasing population in both Peninsular Malaysia and Sumatra, Indonesia (Chua et al. 2000).

We know that diatom abundance, biovolume and diversity are driven by temperature, light, nutrient availability, grazing and viral lysis (Rousseau et al. 2002; Brussaard 2004; Tan et al. 2004; Lim et al. 2015), and variations in these factors also drive diatom species succession (e.g. Bouvy et al. 2006; Rousseau et al. 2002; Yao et al. 2011; Marić et al. 2012). Since tropical waters are characterized by their relatively high and stable temperature (Lee et al. 2009), observations in tropical region exhibit less influence from the wide temperature fluctuations often observed in higher latitudes. On this basis, we can examine the stability of diatom communities in tropical regions. Moreover, the various states of degradation in the coastal waters along the Straits of Malacca (Chua et al. 2000) provide us with a platform to investigate how diatom may respond to eutrophication.

Although it is essential to understand the ecology of marine diatom and its function in an ecosystem, existing reports on primary producers from the Straits of Malacca are limited to the distribution of phototrophic picoplankton (Lee et al. 2013), primary production measurements (Lee and Bong 2008; Lim et al. 2015) and 'snap shots' of phytoplankton diversity (e.g. Salleh and Tajuddin 2006; Salleh et al. 2008). There is a lack of information on the composition of diatom in these seas, their temporal variation and the environmental drivers for their abundance and diversity.

In this study, we measured diatom abundance, biovolume and diversity over a 2-year period at two stations with upper and lower states of eutrophication (Lee et al. 2009). The 2-year sampling provided a good knowledge of a habitat's natural variability in order to measure any effect of eutrophication. Results from this study also provide a reference point for future comparison.

Materials and methods

Sampling was carried out from January 2010 to December 2011 during high tide at two different stations, i.e.

Klang (3°0' N, 101°24' E) and Port Dickson (2°31' N, 101°47' E) (Fig. 1). Klang station was located at an estuary whereas Port Dickson station represented a coastal water habitat. Seawater sample was collected from the surface (about 0.1 m depth) with precleaned bottles and transported back to the laboratory in a cooler box for further processing.

Surface seawater temperature and salinity at the time of sampling were measured in situ with a conductivity meter (YSI-30, USA) and water transparency was determined as Secchi disc depth. Sample for dissolved oxygen (DO) measurement was fixed in situ, and DO level was determined by the Winkler titration method (Grasshoff et al. 1999). We also concentrated the sample in situ using a plankton net with a 20- μ m pore size mesh filter. The concentrated sample (> 20 μ m fraction) was preserved immediately with 4% Lugol's iodine and kept at 4 °C until observation using an inverted microscope (Thronsen 1978).

In the laboratory, seawater sample was filtered through precombusted (500 °C for 3 h) GF/F filters. The filters were kept for total suspended solids (TSS) and chlorophyll *a* (Chl *a*) determination whereas the filtrate was kept frozen at - 20 °C until nutrient analysis [ammonium (NH₄), nitrate + nitrite (NO₃ + NO₂), silicic acid (Si(OH)₄) and phosphate (PO₄)]

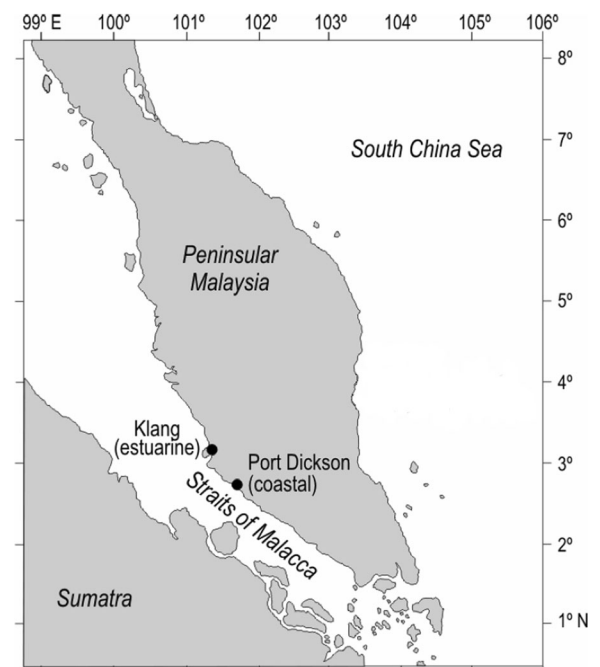


Fig. 1 Location of the sampling stations: Klang (3°0' N, 101°24' E) and Port Dickson (2°31' N, 101°47' E)

(Parsons et al. 1984). TSS were measured as GF/F filter weight increase after drying at 50 °C for a week whereas Chl *a* was extracted overnight (> 16 h) with 90% ice-cold acetone at -20 °C. Chl *a* was then determined via a 1-cm light path cuvette using a spectrophotometer (Hitachi U-1900, Japan). Chl *a* was then calculated according to the tri-chromatic equation with absorbance at wavelengths of 630, 647, 664 and 750 nm (Parsons et al. 1984):

$$C = 11.85 (Abs_{664} - Abs_{750}) - 1.54 (Abs_{647} - Abs_{750}) - 0.08 (Abs_{630} - Abs_{750})$$

Where, Chl *a* ($\mu\text{g L}^{-1}$) = $(C \times \text{volume of acetone}) / (\text{volume of sample filtered} \times 10)$.

Enumeration and identification of diatom were carried out by observing a sedimentation chamber through an inverted microscope (IX51 Olympus, Japan). In this study, up to 3 L of the sample was concentrated before microscopy. The detection limit was 55 cells L^{-1} whereas for logistic reasons, diatom was only identified to the genus level (Salleh and Tajuddin 2006). We also measured the biovolume of each diatom by its morphometric characteristic (e.g. diameter or width and length) using the Olympus Cell D imaging system (Japan). Although diatom biovolume is affected by the fixation step (Menden-Deuer et al. 2001), and the measurement of biovolume is laborious, we used systematic approaches to cell size and biovolume measurements (Leblanc et al. 2012). In this study, six groups of commonly observed morphology (Hillebrand et al. 1999) were used. This approach does not reflect the diverse diatom shapes but is more of a practical concession (Vadrucci et al. 2007). We also did not correct for inert cell structures such as vacuoles. Volume was calculated for each cell and the total biovolume per sample was the sum of the estimated cell volume for each species. The estimated diatom biovolume was then converted into carbon units using the following equation (Menden-Deuer and Lessard 2000): $C = 0.288 \times \text{biovolume}^{0.811}$ where C is pgC per cell and biovolume is cubic micrometre. In this study, Shannon index (H') (Shannon and Weaver 1949) was calculated based on the proportion of the biomass of each genus whereas Shannon's equitability or evenness (E_H) was calculated by dividing H' with the natural logarithm of the total number of genera (Sugie and Suzuki 2015).

All values were reported as mean \pm standard deviation (S.D.) unless stated otherwise. Diatom count was log-transformed before statistical analysis in order to meet parametric assumptions of equality of variance

and normal distribution. Student's *t* test was used to compare the environmental parameters between Klang and Port Dickson whereas analysis of similarity (ANOSIM) was used to compare the diatom community structure from the two stations. In order to analyse the diatom community profiles over time, a cluster analysis based on the unweighted pair group method with arithmetic mean (UPGMA) with distance via Bray-Curtis index was used. The stability of the resulting dendrogram was tested by bootstrapping 1000 times. ANOSIM and cluster analyses were carried out with the software PAST (Hammer et al. 2001) whereas correlation and linear regression analyses were carried out via the spreadsheet Microsoft Excel (MS Office 2003, USA) according to Zar (1999).

Results

Environmental characteristics

Both stations had relatively high and stable surface seawater temperatures (> 29 °C) (Table 1). The estuarine waters at Klang exhibited strikingly different environmental characteristics relative to the coastal waters of Port Dickson. At Klang, the average salinity was lower ($t = 2.45$, $df = 30$, $p < 0.05$) as there was freshwater influence. Klang waters were also more eutrophic with lower DO ($t = 10.02$, $df = 32$, $p < 0.001$), lower transparency ($t = 6.22$, $df = 31$, $p < 0.001$) and higher TSS ($t = 3.08$, $df = 36$, $p < 0.001$). With the exception of PO_4 , nutrient concentrations at Klang were also higher [NH_4 ($t = 3.92$, $df = 23$, $p < 0.001$), $\text{NO}_3 + \text{NO}_2$ ($t = 9.49$, $df = 35$, $p < 0.001$) and Si(OH)_4 ($t = 4.55$, $df = 26$, $p < 0.001$)] (Supplement Figure 1).

Diatom abundance and biomass

In this study, Chl *a* fluctuated from 0.20 to 4.61 $\mu\text{g L}^{-1}$ ($1.50 \pm 1.22 \mu\text{g L}^{-1}$) at Klang except on Sep 2011 and Nov 2011 when Chl *a* concentrations were 26.31 and 11.97 $\mu\text{g L}^{-1}$, respectively (Fig. 2). In contrast, Chl *a* concentration at Port Dickson was relatively stable and ranged from 0.14 to 2.76 $\mu\text{g L}^{-1}$ ($1.50 \pm 0.72 \mu\text{g L}^{-1}$). Diatom abundance at Klang ranged from 0.2×10^4 to 21.7×10^4 cells L^{-1} whereas diatom abundance at Port Dickson was about one order lower from 0.9×10^3 to 41.3×10^3 cells L^{-1} .

Table 1 Environmental conditions at Klang and Port Dickson (mean \pm S.D). Significance of differences between the sampling sites

	Klang	Port Dickson
Temperature ($^{\circ}$ C)	29.5 \pm 0.8	29.5 \pm 1.1
Salinity (‰)*	24.2 \pm 6.0	27.1 \pm 2.6
DO (μM)**	150 \pm 30	210 \pm 15
Secchi depth (m)**	0.62 \pm 0.16	1.09 \pm 0.37
TSS (mg L^{-1})**	62 \pm 15	51 \pm 10
NH ₄ (μM)**	17.49 \pm 20.93	1.29 \pm 0.97
NO ₂ + NO ₃ (μM)**	8.26 \pm 4.08	1.20 \pm 1.04
SiO ₄ (μM)**	24.65 \pm 18.27	9.02 \pm 4.55
PO ₄ (μM)	1.09 \pm 1.16	0.67 \pm 0.73

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

We also measured diatom biovolume in order to estimate the contribution of diatom towards the carbon pool. Table 2 shows the diatom genera observed within each morphological group adopted in this study. The top five genera that contributed to diatom biovolume were the same at both Klang and Port Dickson but their order of importance differed. The top five diatom genera were *Chaetoceros* spp., *Pleurosigma* spp., *Skeletonema* spp.,

Navicula spp. and *Thalassionema* spp. These diatoms accounted for about $85 \pm 19\%$ and $73 \pm 25\%$ of the total biovolume at Klang and Port Dickson, respectively. Total diatom biovolume fluctuated over a wide range (coefficient of variation, CV > 162%) at both Klang and Port Dickson (0.02 to $99.3 \times 10^7 \mu\text{m}^3 \text{L}^{-1}$ and 0.07 to $41.4 \times 10^8 \mu\text{m}^3 \text{L}^{-1}$, respectively) and averaged $1.02 \pm 2.09 \times 10^8 \mu\text{m}^3 \text{L}^{-1}$ and $6.33 \pm 10.28 \times 10^8 \mu\text{m}^3 \text{L}^{-1}$, respectively. The average diatom biomass calculated from biovolume was $4.59 \pm 7.11 \mu\text{g C L}^{-1}$ at Klang and $15.22 \pm 18.82 \mu\text{g C L}^{-1}$ at Port Dickson. The top five genera accounted for $86 \pm 19\%$ of the total diatom biomass at Klang with *Skeletonema* spp. making up for $60 \pm 32\%$ whereas at Port Dickson these five genera made up $71 \pm 25\%$ of the total diatom biomass with *Chaetoceros* spp. contributing $48 \pm 30\%$ (Fig. 3).

Cellular biovolume for the diatoms observed in this study ranged from 20.14 to $1.65 \times 10^7 \mu\text{m}^3 \text{cell}^{-1}$ and was, on average, higher at Port Dickson. For the five predominant genera, average cell biovolumes of *Chaetoceros* spp. ($p < 0.05$), *Skeletonema* spp. ($p < 0.001$) and *Thalassionema* spp. ($p < 0.01$) were significantly higher at Port Dickson but not for *Pleurosigma* spp. ($p > 0.10$) and *Navicula* spp. ($p > 0.10$) (Fig. 4).

Diatom composition and diversity

A total of 28 diatom genera were recorded in this study with 24 genera observed in Klang and 27 genera in Port Dickson. Even though diatom species were not determined in this study, genus richness was higher at Port Dickson. Among the diatoms observed at both Klang and Port Dickson, *Thalassiosira* spp. were unique for Klang waters whereas *Helicotheca* sp., *Diploneis* spp. and *Asteromphalus* spp. were unique for Port Dickson. Using the Bray-Curtis coefficient, we showed via ANOSIM that the diatom community structure at both stations was different ($R = 0.471$, $p < 0.001$). Diatom community at Klang ($H' = 0.77 \pm 0.48$) was also less diverse than that at Port Dickson ($H' = 1.40 \pm 0.67$) ($t = 3.64$, $df = 40$, $p < 0.001$). Although diversity was different between the two stations, E_H at both Klang ($E_H = 0.46 \pm 0.18$) and Port Dickson ($E_H = 0.46 \pm 0.23$) were similar.

In this study, cluster analysis was used to investigate the temporal variation of diatom community profiles. The results revealed no clades at Port Dickson (Fig. 5).

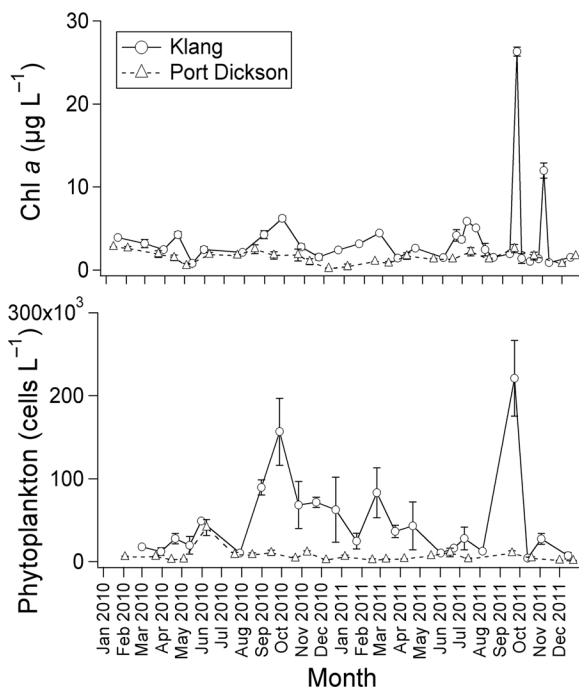


Fig. 2 Top panel Temporal variation of Chl *a* ($\mu\text{g L}^{-1}$) at Klang and Port Dickson. Bottom panel Temporal variation of diatom abundance (cells L^{-1}) at Klang and Port Dickson. The error bar for standard deviation is shown unless smaller than the symbol

Table 2 Morphological groups in this study and the phytoplankton genera identified in each group

Morphological groups	Genus	Morphological groups	Genus
Cylinder + two half spheres	<i>Corethron</i> spp. <i>Skeletonema</i> spp.	Prism on parallelogram-base	<i>Nitzschia</i> spp. <i>Pleurosigma</i> spp. <i>Pseudo-nitzschia</i> spp.
Rectangular box	<i>Thalassionema</i> spp.	Cylinder	<i>Asteromphalus</i> spp. <i>Azpeitia</i> spp. <i>Bacteriastrum</i> spp. <i>Coscinodiscus</i> spp. <i>Guinardia</i> spp. <i>Leptocylindrus</i> spp. <i>Melosira</i> spp. <i>Rhizosolenia</i> spp. <i>Thalassiosira</i> spp.
Elliptic + prism	<i>Biddulphia</i> spp. <i>Chaetoceros</i> spp. <i>Diploneis</i> spp. <i>Eucampia</i> spp. <i>Helicotheca</i> sp. <i>Hemialus</i> spp. <i>Meuniera</i> sp. <i>Navicula</i> spp. <i>Odontella</i> spp. <i>Striatella</i> spp.	Prism on triangle	<i>Asterionellopsis</i> spp. <i>Lithodesmium</i> spp. <i>Ditylum</i> spp.

None of the early branches were stable as their bootstrap values were <30%. One outgroup (16 Jun 2011) was characterized by an absence of *Skeletonema* spp., increased *Guinardia* spp. and reduced *Chaetoceros* spp. In contrast, diatom communities at Klang formed two main clades and one outgroup (5 Aug 2011). Similar to the observation at Port Dickson, the outgroup at Klang was due to an absence of *Skeletonema* spp. but with an increased *Nitzschia* spp. At Klang, the two clades were well supported with bootstrap values >60%. Although some successive samplings clustered in different clades, we found a few periods of successive samplings that were grouped in the same clade.

One of the clades was at a similarity index of 0.37 and made up of diatom community profiles during the drier months of May–June–July 2010 and June–July 2011. The diatom communities during these months were predominated with *Skeletonema* spp. with counts in the range of 1.5–13 × 10³ cells L⁻¹. Concurrently, we observed an increase in both *Chaetoceros* spp. and *Leptocylindrus* spp. Another clade was at a similarity index of 0.56, and the diatom communities in this clade were from the intermonsoon periods Aug–Sep–Oct–Nov 2010 and Feb–March–April 2011. This clade was characterized by higher *Skeletonema* spp. counts (2.9–

14.6 × 10⁴ cells L⁻¹) and was also characterized by increased *Thalassiosira* spp., *Thalassionema* spp., *Navicula* spp. and *Melosira* spp. Although the diatom community profiles for the following sampling days—23 Dec 2010, 3 Nov 2011, 21 Apr 2010 and 23 Sep 2011—were also in this clade, contiguous sampling for these months was placed in another clade. Our analyses suggested that there was still some uncertainty to how the diatom community was structured at Klang but the presence of *Skeletonema* spp. was an important factor.

Discussion

Environmental characteristics

The results of the physico-chemical parameters measured in this study reflected the eutrophication that is prevalent at the Klang estuary (Lee et al. 2009) and is driven by higher nutrient levels in the river (Lim et al. 2015). The spike in Chl *a* concentration at Klang is a regular phenomenon that usually occurs when there is more rainfall for example during the intermonsoon and wet seasons. At Port Dickson, a relatively stable Chl *a*

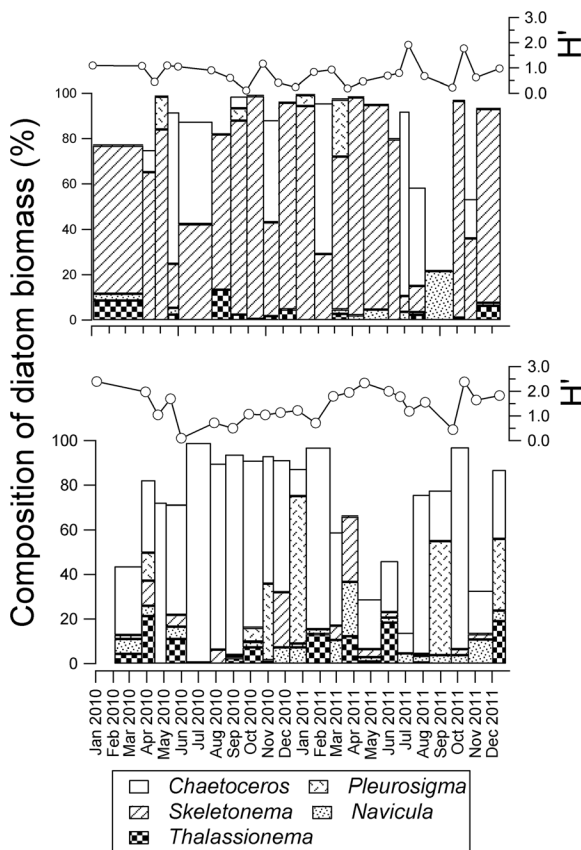


Fig. 3 Composition of diatom biomass (%) as contributed by the top five genera (*Chaetoceros* spp., *Skeletonema* spp., *Thalassionema* spp., *Pleurosigma* spp. and *Navicula* spp.) at Klang and Port Dickson. The Shannon index (H') is also shown

regime was observed which probably reflected the absence of any large riverine or anthropogenic input.

Diatom abundance and biomass

Diatom abundance was higher at Klang ($t = 4.25$, $df = 38$, $p < 0.001$) but was predominantly of a smaller size. Abundance of smaller diatom is usually higher as the specific growth rate of diatom decreases as size increases (Sarhou et al. 2005; Marañón et al. 2013). We found that although diatom abundance at Klang was about one order higher than at Port Dickson, the higher abundance did not compensate for the size deficit. As a result, diatom biomass at Klang was lower than that at Port Dickson ($t = 2.54$, $df = 28$, $p < 0.05$). Diatom biomass also correlated with Chl *a* at both Klang ($R^2 = 0.305$, $p < 0.05$) and Port Dickson ($R^2 = 0.225$, $p < 0.05$) which was expected as diatoms carry out photosynthesis (Round et al. 1990).

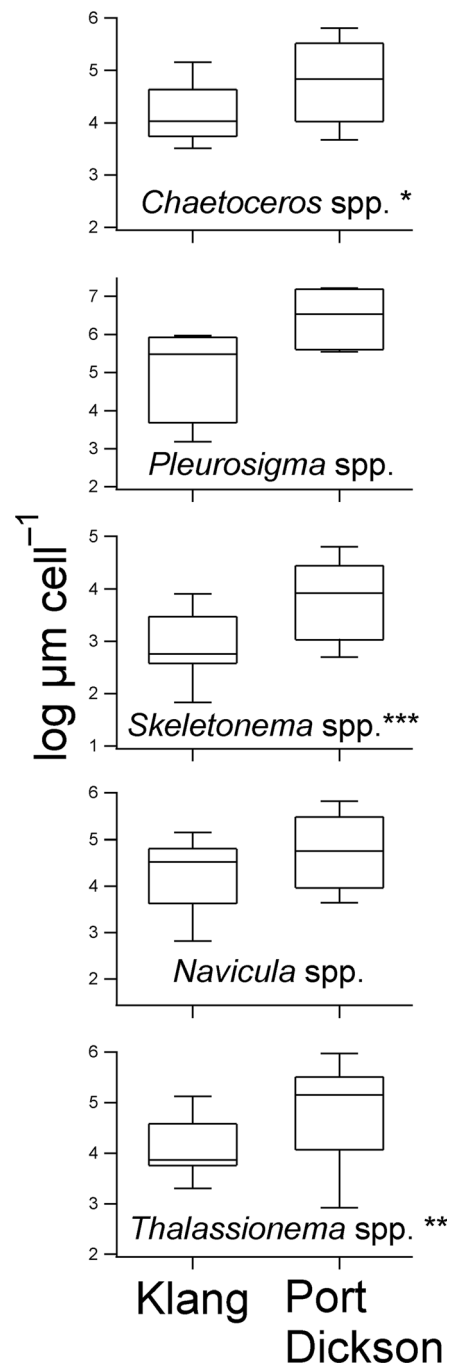


Fig. 4 Box-and-whisker plots showing the range and the median of diatom biovolume ($\log \mu\text{m}^3 \text{cell}^{-1}$) of *Chaetoceros* spp., *Pleurosigma* spp., *Skeletonema* spp., *Navicula* spp. and *Thalassionema* spp. measured at Klang and Port Dickson. Asterisks placed after the diatom genera denote the significance of the Student's *t* test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

In this study, we concentrated the diatoms with a 20- μm pore size mesh filter. Although smaller diatoms

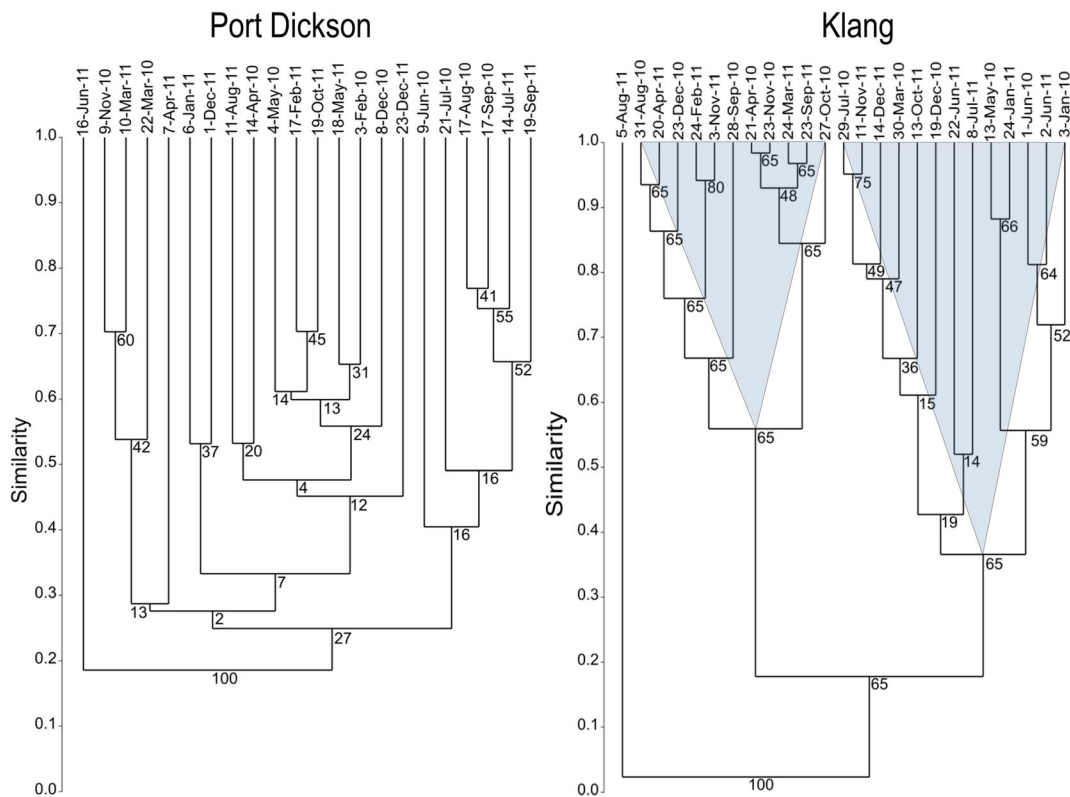


Fig. 5 Cluster analysis of the diatom community structure obtained during samplings at Port Dickson (*top panel*) and Klang (*bottom panel*) using Bray-Curtis index of similarity. The clades observable at Klang are highlighted as *triangles*

(e.g. Sugie and Suzuki 2015) may be overlooked, diatoms are mostly dominated by microphytoplankton (> 20 μm) in coastal waters (Thomas et al. 2013). Moreover, a recent sampling (May and June 2017) at both Klang and Port Dickson revealed diatoms in the < 20 μm fraction were at 8 and 21% of total diatom abundance, respectively (unpublished data). The exclusion of the < 20 μm fraction was probably not enough to change the more than one-order difference in diatom abundance and the conclusion that diatom biomass in Port Dickson was higher.

The diatom biomass measured in this study filled an obvious data gap. From a comprehensive analysis of existing diatom data, only a small fraction has a biovolume or biomass component of which none was from the Straits of Malacca (Leblanc et al. 2012). Average diatom biomass estimated at Klang and Port Dickson was lower than the global mean diatom biomass of 141.19 μg C L⁻¹ (Leblanc et al. 2012). However, the diatom biomass estimated in this study fluctuated over a wide range where the CV was 160 and 124% for Klang and Port Dickson, respectively.

As Chl *a* concentrations at both Klang and Port Dickson were essentially the same, the higher diatom biovolume and biomass at Port Dickson suggested a difference in the Chl *a* content per diatom. This was reasonable due to the different genera predominant at Klang and Port Dickson. For example, the smaller *Skeletonema* spp. (mean = 2.3 × 10³ μm³ cell⁻¹) were predominant at Klang whereas the larger *Chaetoceros* spp. (mean = 2.0 × 10⁵ μm³ cell⁻¹) were the main diatoms at Port Dickson. We also observed that even within the same genera, diatoms were generally larger at Port Dickson (e.g. *Chaetoceros* spp., *Skeletonema* spp. and *Thalassionema* spp.). Although the difference in biovolume of the same genera could suggest the presence of different species at different stations, this hypothesis was not investigated further.

We also observed from correlation analyses that nutrients could be an important environmental driver for diatom abundance. Diatom abundance correlated positively with NH₄ (R² = 0.212, df = 43, p < 0.01), Si(OH)₄ (R² = 0.247, df = 43, p < 0.001) and NO₃ + NO₂ (R² = 0.263, df = 43, p < 0.001). Although causality

cannot be inferred from correlation, the influences of nutrients on diatom abundance are well documented and generally accepted (e.g. Underwood et al. 1998; Rousseau et al. 2002; Larsen et al. 2004). Multiple linear regression analysis for diatom abundance against the above factors was significant but could only explicate 34% of the variation in diatom abundance [\log diatom abundance = $0.011 \text{ NH}_4 - 0.003 \text{ Si(OH)}_4 + 0.050 (\text{NO}_3 + \text{NO}_2) + 3.652$ ($F = 6.87$, $df = 43$, $p = 7.72 \times 10^{-4}$)]. Hence, there remained other factors that could have affected diatom abundance in this study. One possible factor is grazing which is coupled to phytoplankton production at Klang and Port Dickson (Lim et al. 2015). Viral lysis may also be important for diatom abundance (Brussaard 2004) but was not investigated in this study.

Diatom composition and diversity

Diatom diversity at Port Dickson was significantly higher and its most abundant genus (*Chaetoceros* spp.) made up a small proportion of the total diatom abundance. In contrast, diatom diversity was persistently lower at Klang, and *Skeletonema* spp. made up more than three quarters of the total diatom abundance. *Skeletonema* spp. are small in size and exhibit rapid response to favourable environmental conditions (Arauzo and Cobelas 1994). As the riverine input at Klang is known to bring nutrients to the estuary in short pulses (Lim et al. 2015), this would probably explain why *Skeletonema* spp. accounted for most of the variation in diatom abundance at Klang and were also the key organisms when the Chl *a* concentration spiked.

Lower diatom diversity is driven by increased eutrophication (Underwood et al. 1998). In this study, proxies for eutrophication included reduced transparency and increased nutrient concentration. Univariate correlation analyses using data from both Klang and Port Dickson showed strong positive correlations between diatom diversity and Secchi disc depth ($R^2 = 0.273$, $df = 40$, $p < 0.001$) whereas diatom diversity was inversely correlated with the NH_4 ($R^2 = 0.175$, $df = 40$, $p < 0.01$), $\text{NO}_3 + \text{NO}_2$ ($R^2 = 0.146$, $df = 40$, $p < 0.01$), Si(OH)_4 ($R^2 = 0.092$, $df = 40$, $p < 0.05$) and PO_4 ($R^2 = 0.219$, $df = 40$, $p < 0.01$) levels (Fig. 6). By using multiple regression analysis, the linear regression equation is as follows: $\text{Shannon} = 0.504 \text{ Secchi} - 0.016 \text{ NH}_4 + 0.005 \text{ Si(OH)}_4 - 0.017 (\text{NO}_3 + \text{NO}_2) - 0.302 \text{ PO}_4 + 1.009$ ($F = 5.34$, $df = 40$, $p = 9.78 \times 10^{-4}$). The regression

model explicated 42% of the Shannon index variation. Our results suggested increased eutrophication at Klang reduced diatom diversity, which concurred with those of Underwood et al. (1998) and Huang et al. (2004).

The predominant *Skeletonema* spp. at Klang also drove the clustering pattern of the diatom community. Each clade at Klang was delineated by lower and higher abundance of *Skeletonema* spp. which in turn occurred during the dry and wet months, respectively. The

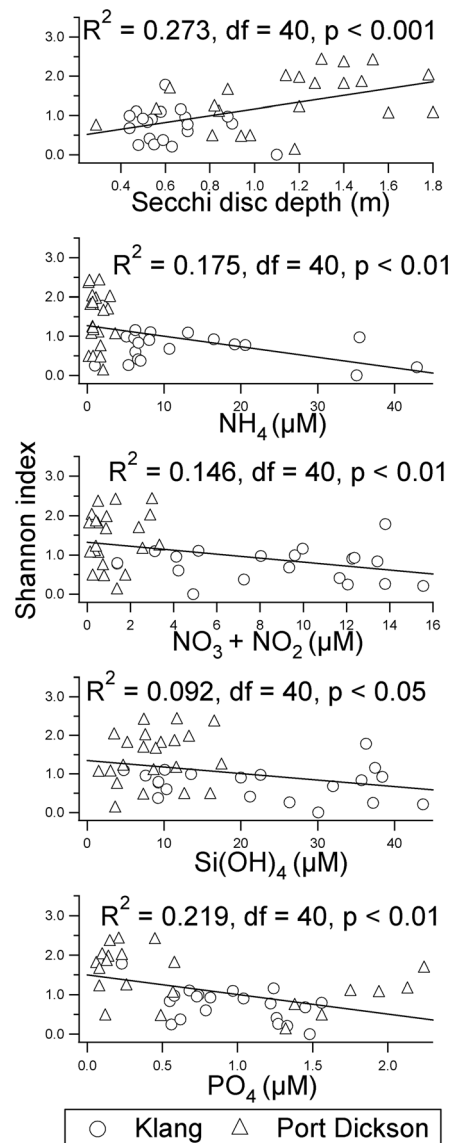


Fig. 6 Multiple univariate correlations between Shannon index and Secchi disc depth (m), Si(OH)_4 (μM), NH_4 (μM), $\text{NO}_3 + \text{NO}_2$ (μM), Si(OH)_4 (μM) and PO_4 (μM) for both Klang (circle) and Port Dickson (triangle). Correlation index and regression lines are also shown

predominance of *Skeletonema* spp. at Klang also affected the diatom diversity where an increase in *Skeletonema* spp. coincided with a decreasing Shannon's diversity index ($R^2 = 0.461$, $df = 20$, $p < 0.001$). Therefore, *Skeletonema* spp. were an important factor in structuring the diatom community at Klang.

We also found that *Skeletonema* spp. abundance correlated significantly with Si(OH)_4 ($R^2 = 0.263$, $df = 21$, $p < 0.05$) alone. Secchi disc depth also did not correlate with *Skeletonema* spp. This relationship is tenable as *Skeletonema* spp. require Si(OH)_4 for building its silica shell (Round et al. 1990). Although other diatoms also uptake Si(OH)_4 for their silica shell formation, Paasche (1973) has shown that among five different diatom genera, *Skeletonema costatum* has the ability to use low levels of Si(OH)_4 . Smaller diatoms are also known for their affinity for nutrient uptake due to the difference in surface area to cell volume ratio and the size of diffusive boundary layer (Sarthou et al. 2005; Sunda and Hardison 2010). Although seasonal variability in temperature, light and nutrient availability often plays a major role in structuring diatom community (e.g. Rousseau et al. 2002; Larsen et al. 2004; Yao et al. 2011), in this study, we showed how Si(OH)_4 could influence *Skeletonema* spp. abundance which in turn affected the diatom community diversity and profile.

Reduced diversity or prevalence of a single species can affect community stability by decreasing the variety of responses or compensation from other species (Vasseur and Gaedke 2007). Therefore, the diatom community at Klang is more at risk from environmental changes. A decrease in diversity is one of the threats of continuous eutrophication in coastal waters (Rabalais et al. 2009), and this scenario is possible if the trend of increasing eutrophication is not reversed in Straits of Malacca or other nearshore coastal waters.

Conclusions

By sampling two habitats with different states of eutrophication, we observed that diatom abundance was higher whereas diatom diversity was lower in the more eutrophic Klang waters. The average diatom biovolume and biomass were also lower at Klang. We showed how nutrients and water transparency were possible drivers for diatom diversity and how Si(OH)_4 affected *Skeletonema* spp. at Klang, which in turn affected the diatom community structure. Our results have implications towards

understanding the dynamics of diatom biomass and community structure in different habitats and highlighted how eutrophication affects diatom diversity and community structure.

Acknowledgements We would like to thank the two anonymous reviewers whose comments have greatly improved this manuscript. This research was supported by the University of Malaya under Grant UM.C/625/1/HIR/050 and RP019A-16SUS; Ministry of Science, Technology and Innovation under Grant eScience 04-01-03-SF0671; and Ministry of Education under HiCoE Grant IOES-2014D.

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