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The Photophysiology of Benthic Diatoms in the Intertidal Flats of Pulau Pinang (Malaysia)

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ABSTRACT

The *in-situ* photosynthetic activity in tropical intertidal benthic diatom in response to environmental variation was assessed in this study by measuring chlorophyll fluorescence. The investigation was carried out during the lowest tide in January (non-rainy day) and February 2013 (post-rainy day) at two sampling sites (A and B) from each selected location (Pantai Jerejak, Teluk Bahang and Tanjung Bungah, Pulau Pinang, Malaysia). Samples of surface sediment (top 0.5 cm) were collected, and chlorophyll *a* extracted as biomass estimation. Assessments of the photosynthetic activity of benthic diatoms were made using a pulse-amplitude modulated (PAM) fluorometer. Fifty-three species were identified, representing 27 genera from the three studied locations. Both locations showed similarities in species diversity and abundance. Two-way ANOVA showed no significant differences ($p = 0.430$) in species richness (Margalef Index) among sampling locations, with an average value of 6.33±0.247. Both intertidal flats were dominated by *Cocconeis*, *Navicula, Actinoptychus,* and *Diploneis*. The community has low maximum quantum yields, F_v/F_m (ranging from 0.170 to 0.340) and is often light-limited (Photoacclimation Index, E_k, ranging from 67.96 to 236.71 µmol photons m^{-2} s^{-1}). The relative electron transport rate (rETRmax) was low, with

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values ranging from 3.45 to 35.51 across three sampling locations. Fluctuation in salinity has caused a decrease in photosynthetic activity. This study suggests that the low values indicated a poorly adapted benthic microalgal community that is constantly light-limited. However, time-series data is needed to determine the ability of these communities to adapt to the changing environment.

Keywords: Chlorophyll *a*, fluoresces, intertidal, microalgae, microphytobenthos, PAM

INTRODUCTION

Benthic diatoms play an essential role in marine benthic environments by providing an adequate food supply for invertebrate grazers and stabilising the sediment substrate. The function of intertidal benthic diatom and species composition shows a strong relationship with environmental factors, mainly sediment grain size composition, tidal exposure, salinity, light availability, temperature, and nutrients (Dalu et al., 2016; Du et al., 2016). Among these factors, sediment characteristics are commonly used to explain variations in abundance and composition in tropical intertidal flats. It is noted that diatoms tend to dominate muddy sediments, while sandy sediments are overwhelmed by the mixed composition of cyanobacteria, diatoms, and euglenids (Ribeiro et al., 2013). In contrast, sediment with high silt content is known to favour high species diversity and larger diatoms (Grinham et al., 2011). Benthic diatoms are divided into two groups according to their colonisation substratum, epipelon, and epipsammon (Round et al., 1990). The epipelon consists of free-living diatoms that can move through muddy sediments. In contrast, the epipsammon comprises smaller, non-motile diatoms attached to the particles of sandy sediments.

In addition to sediment type, light and temperature are among the factors influencing diatom biomass and photosynthetic capacity in shallow coastal environments (Mitbavkar & Anil, 2002). For instance, differences in sediment size can influence light quality. Whereby, in muddy sediment, light is fully attenuated at a depth of a fraction of a millimetre, and in sediment or larger grain size, it can penetrate deeper (Cartaxana et al., 2011; Vieira et al., 2013). In tropical intertidal flats, the light intensity on the sediment surface varies from less than 50 μmol $m^{-2}s^{-1}$ at high tide to more than 1800 μmol $m^{-2}s^{-1}$ at low tide, and the exposure time can be as long as 6–7 hours depending on the tidal cycle (Salleh & McMinn, 2021). Besides, during low tide exposure, sediment temperature could vary from 25 to 40°C. In addition to light and temperature, environmental cues can rapidly vary to an extreme in intertidal environments when changes in salinity occur due to desiccation and heavy rainfall. Such rapid changes in these intense environmental factors can drive the benthic diatom to develop many acclimation mechanisms to reduce the stress of highly variable light intensity. Severe light stress could impair the Photosystem II (PSII) of diatom and cause a reduction in the quantum yield. However, many can migrate vertically, hence positioning themselves within the sediment at a depth that provides shading from excess irradiance and provides an optimal light environment for their photosynthetic activities (Cartaxana et al., 2011; Perkins et al., 2010b), hence avoiding severe photoinhibition and maintaining their presence in the intertidal habitat. In addition, diatoms can activate their photoprotective mechanism (activation of the xanthophyll cycle) when exposed to saturating irradiance. A decrease in the maximum quantum yield (F_v/F_m) in benthic diatoms is commonly observed at low tide but increased at high tide. Their ability to subsequently recover during high tide suggests that down-regulation of photosynthesis and up-regulation of photoprotection occurs, preventing PSII damage (Salleh & McMinn, 2021). Fluctuations in light intensity and temperature are often considered the two major forcings of the photosynthetic activities in diatoms (Béchet et al., 2017).

Since most chlorophyll fluorescence originates from PSII, and PSII is the most sensitive component of photosynthesis, chlorophyll fluorescence is an ideal tool to measure the short-term response of photosynthetic efficiency to changes in environmental stressors. The introduction of Pulse Amplitude Modulated (PAM) fluorometer has allowed researchers to study the activity of PSII, based on the direct determination of variable chlorophyll *a* fluorescence index (Consalvey et al., 2005; Perkins et al., 2002; Serôdio et al., 2012). This method is rapid and non-destructive and allows in-situ assessment of photoacclimation by providing rapid collection of a suite of photophysiological parameters which can be used to assess the physiological status or as a measure of stress (Consalvey et al., 2005; Perkins et al., 2010a). Furthermore, the Rapid Light Curves (RLCs) (see Ralph, & Gademann, 2005) can be constructed to understand the current photosynthetic capacity and also responses over a broad range of ambient light conditions (Consalvey et al., 2005).

Pulau Pinang is in the Northern Straits of Malacca and is Malaysia's well-developed state. The coast of Pulau Pinang has changed in the last decade due to vast developments and land reclamation. The coastal area's water quality has significantly deteriorated due to excessive organic release from industrial, residential, mariculture activities and active coastal developments. Albeit this situation, the coastal intertidal flats of Pulau Pinang pose as one of the significant benthic habitats for marine communities and fishing grounds for coastal fisheries for nearby fishing communities. Here, using a PAM fluorometer, we examined the response of MPB to salinity change in the intertidal flats of Pulau Pinang post-rainfall at low tide. We aim to determine the physiological response of MPB to salinity change, testing the hypothesis that salinity change due to rainfall inhibits photosynthesis. In addition, limited data have been available on benthic diatom communities in Malaysia, especially Pulau Pinang. Thus, this paper focuses on the photosynthetic health, species composition, and photosynthetic characteristics of benthic diatom from three intertidal flats, which are Pantai Jerejak (industrial and residential), Tanjung Bungah (residential and tourism), and Teluk Bahang (fishing and aquaculture).

MATERIALS AND METHODS

Site Description and Sampling Procedure

Pulau Pinang is in the Northern Straits of Malacca (NSoM) (Figure 1). The area has a humid, tropical climate with daily temperatures between 23–32°C (Darif et al., 2016). Sampling was conducted at intertidal flats of Pantai Jerejak, PJ (5°20'35.6" N 100°18'43.9" E), Teluk Bahang, TB (5°27'46.05" N, 100°12'11.48" E), and Tanjung Bungah, TJB (5°28'3.94''N, 100°16'42.53''E) at low tide when most of the sediment was exposed during tidal emersion. To better understand the surrounding environment at each sampling location, two random sites were sampled at least 100 m apart (labelled as Site A and B). The sampling period was established to coincide with a spring tide to maximise tidal exposure; hence porewater was collected for analysis (nutrients, pH and salinity). The sampling was conducted once in January and February, whereby heavy rainfall was observed before sampling in February, thus allowing us to determine the impact of salinity change on the photosynthetic activities of MPB.

Pantai Jerejak (PJ) is located on the east coast of Pulau Pinang. The coast of Pantai Jerejak is currently undergoing rapid developments and land reclamation along its coast. Besides, it is surrounded by residential areas, industrial zones, and the aquaculture industry from Peninsular Malaysia and Pulau Pinang. A Free Industrial Zone (FIZ) of Bayan Lepas was established in 1976. It is consisted of four stages to accommodate various light and massive factories and is located close to the study area. Discharges from factories are drained into canals, the Keluang River, and the surrounding coastal area.

Tanjung Bungah (TJB), situated in the Northwest of Pulau Pinang and is also a moderately exposed sandy beach. The sandy beach of Tanjung Bungah is known to the locals for water sports activities. Tanjung Bungah is more of a residential area than Pantai Jerejak, where houses and resorts are built to accommodate the increase in the human population in that area. The sampling site is located within the proximity of a fishing village. This coastal area houses at least 50 small-scale fishing boats (<40GRT), which operates by traditional fishers within the coast (0–5 nautical miles, Zone A) of Tanjung Bungah and adjacent areas.

Teluk Bahang (TB) is in the north of Pulau Pinang. In addition, it is surrounded by the Penang National Park and is famous for its fishing landing area for various fishing vessels and the aquaculture industry with at least ten fish cages. Apart from being the largest fishing village in Pulau Pinang, the coastal area of Teluk Bahang has undergone minimal development without any reclamation by comparison to Pantai Jerejak and Tanjung Bungah.

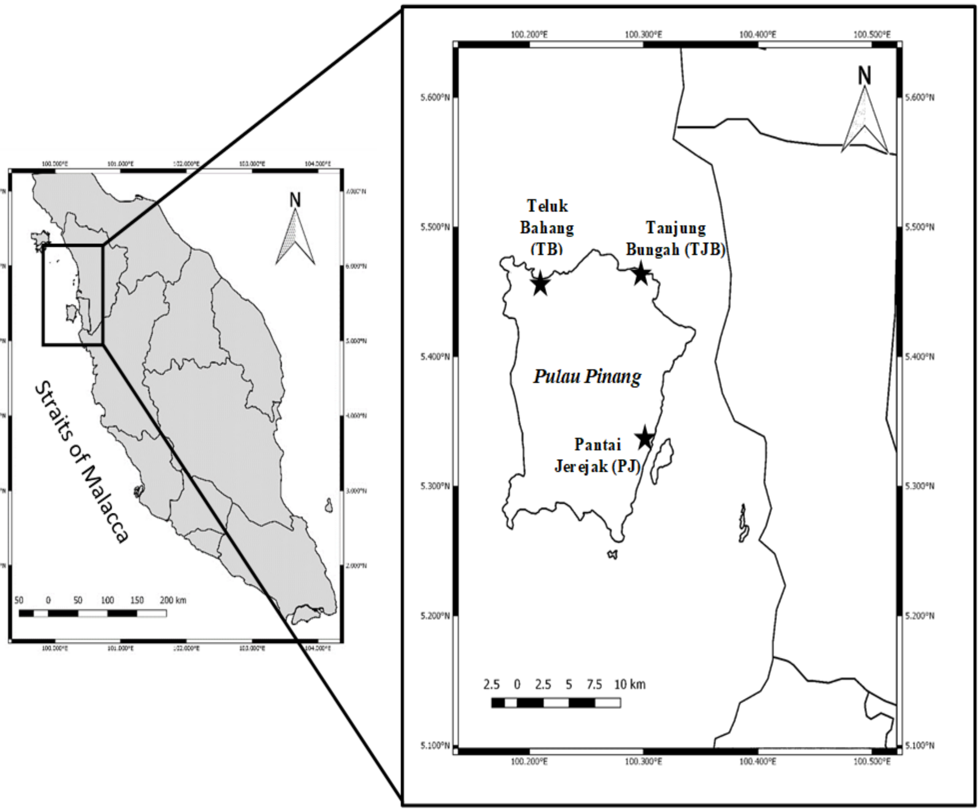


Figure 1. Sampling sites (★) on the coast of Pulau Pinang, Malaysia

Benthic Diatom Sampling and Processing

For species composition, benthic diatom samples were collected from the top 0.5 cm of sediment using a 15 mm diameter hand-pushed mini corer from the intertidal zone at each site (Jordan et al., 2010). The collected samples were stored in a polyethylene container and preserved using Lugol's iodine solution until the sample analysis (Dalu et al., 2016). The sample digestion was done using 10% hydrogen peroxide solution following Cunningham et al., (2003) and was identified and counted using the phase-contrast light microscope (Olympus BX41) at ×1000 under oil immersion. Slides containing between 200 and 400 frustules were used to identify and count diatoms (in percentage relative abundance) (Dalu et al., 2016). Due to limited taxonomic information on tropical benthic diatoms, identification was mainly based on appropriate references from the temperate areas (Round et al., 1990; Stidolph, 1980; Witkowski, 2000).

Benthic Chlorophyll *a* Biomass

Benthic chlorophyll *a* was measured as a proxy for algal biomass. The chlorophyll biomass was determined following Jordan et al. (2010). For chlorophyll *a* biomass, a 30 mm diameter clear polycarbonate cores were manually pushed into the sediment and were stopped using a rubber bung and immediately returned to the temporarily established working place. The sediment for chlorophyll *a* analysis was placed in an ice-filled, light-proof container and immediately transferred to the laboratory. The top 0.5 cm of sediment was re-suspended in 10 ml methanol, thoroughly mixed, and then stored in the dark for 12 hours at 4°C. After the sediment had settled, the solvent was decanted to measure the chlorophyll *a* content using the acidification method (Holm-Hansen & Lorenzen, 1965). A spectrophotometer (UVmini-1240, Shimadzu, JAPAN) with 90% methanol as the blank was used to measure chlorophyll *a* Biomass (Strickland And Parsons 1968; Hing et al. 2012).

Environmental Parameters and Pore Water Nutrients

To determine the environmental and nutrient status at all sampling sites, environmental parameters and nutrients were recorded at low tide during each sampling event. Sediment temperature (˚C) was, measured using a digital direct probe thermometer (Hanna Instrument, USA) and porewater pH was measured using a pH meter (Starter 300, OHAUS, USA). Porewater salinity and sediment surface irradiance (µmol photons m^{-2} s^{-1}) were measured using a digital refractometer (Hanna instrument, USA) and a LI-COR Biosciences LI-205A (USA) light meter, respectively. Determinations of nutrient concentrations such as nitrite (NO_2^- – N/L), nitrate (mg NO_3^- – N/L), ammonia (mg NH_3 – N/L), and ortho-phosphate (mg PO_4^{3-}– N/L) were analysed from 500 mL of pore water (n=3) using standard titration method (Hing et al., 2012; Strickland and Parsons, 1968). At every sampling event, three replicates of sediment cores (5 cm diameter; 1 cm depth) were also collected for sediment grain size determination (Abdullah et al., 2011). Samples for the analysis were wet sieved at 63 µm and 2000 µm mesh size that differentiated them into three different categories (mud, sand and gravel) using standard sieving methods (Folk, 1954).

PAM Chlorophyll Fluorescence Measurements

Using a Pocket Pulse Amplitude Modulation (PAM), we measured the variable fluorescence to determine the benthic diatom community's photosynthetic health. The PAM methodology followed McMinn et al. (2005). This study uses a Pocket-PAM, which might not be like other operating PAMs; care was taken when interpreting the photosynthetic output. Figueroa et al., (2013) noted that the Water-PAM has a lower detection limit (0.025 µg chl *a* L^{-1}) by comparison to the Pocket-PAM (500 µg chl *a* L^{-1}). Hence, the Water-PAM has higher sensitivity than the Pocket-PAM.

Three 30 mm diameter hand-pushed sediment cores were taken for photosynthetic parameter analysis on each sampling occasion. The collected sediment sample was then diluted with filtered seawater, and 10 ml of supernatant was then placed on the Pocket-PAM optical head for measurements. Photomultiplier gain (PM-Gain) settings were set to 2 before each measurement to keep the measures consistent between samples. The minimum (F_o) and maximum (F_m) fluorescence signals were determined on dark-adapted samples. To calculate the maximum quantum yield (F_v/F_m), samples were dark-adapted for 20 minutes by wrapping the containers in foil and placing them in the dark (Consalvey et al., 2005). The F_v/F_m was calculated according to (Schreiber, 2004). $F_v/F_m = (F_m – F_o)/F_m$, where F_v is the difference between F_o and F_m. F_v/F_m values are often used as a sensitive indicator of photosynthetic stress or health status for microalgae (Perkins et al., 2006).

Rapid light curves (RLCs) were taken under software control (Wincontrol, Walz) with 10 s interval duration to eight incremental irradiances at 90, 125, 190, 285, 420, 625, 820, and 1150 μmol photons m^{-2} s^{-1} to obtain values for maximum quantum yield (F_v/F_m), relative electron transfer rate (rETR), photosynthetic efficiency (α) and photoacclimation index (E_k) (Ralph & Gademann, 2005). The rETR was calculated by multiplying the irradiance by the quantum yield measured at the end of that interval. PAR versus rETR curves was described using the non-linear regression curve fitting protocols on SPSS software (SPSS Inc. Version 21.0, IL, USA). RLCs provide an insight into an algae's physiological potential and its ability to adapt its photosynthetic apparatus to rapid changes in light intensities (Ralph & Gademann, 2005).

Data Analysis

Differences in environmental variables, abundance, biomass (benthic chlorophyll-*a* concentration), photosynthetic parameters and community indices of the sampling months and sites were tested through Two-way analysis of variance (ANOVA) followed by post hoc Tukey tests using SPSS V 21.0. Principal component analysis (PCA) was used to explore the major variation patterns in the environmental data set. The non-metric multidimensional scaling (nMDS) was used to classify the samples based on the species composition. Species data were square-root transformed to reduce the effects of dominant taxa, and the Bray-Curtis resemblance measure was applied using the PRIMER 7 software package (Clarke & Gorley, 2015). For each sample, the Shannon diversity index (H'), Species Evenness (Pielou Index), and Species Richness (Margalef Index) were calculated to characterise the species diversity in a community across locations.

RESULTS

Grain Size Composition

The grain size composition at the sampling locations and sites are shown in Figure 2. Sediment grain size was not significantly different ($p > 0.05$) across the sampling locations. The grain size composition was composed of a mixture of mud, sand, and gravel with high sand percentage at all sampling locations. However, In Tanjung Bungah (TJB), sediment composition changed significantly in February after the heavy rainfall. A higher percentage of gravel (84%) was observed in site A compared to January (13%), while the mud percentage increased in site B from 4 to 30% in February.

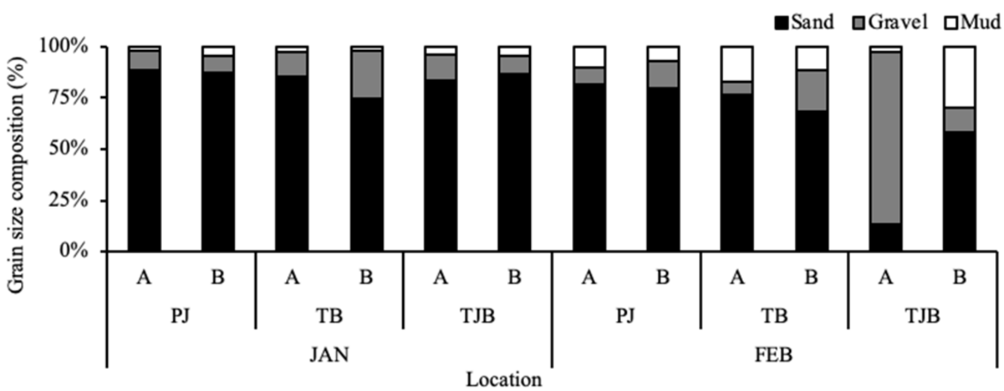


Figure 2. Grain size composition in the studied locations and sites. Grain size fractions were expressed in percentages and consisted of sand, gravel, and mud.

Environmental Parameters and Nutrients

Environmental parameters (porewater salinity and temperature) and nutrients varied significantly ($p < 0.001$) among the sampling locations across the three sampling locations and sites. As sample collection was conducted between 0730 to 0900 hrs in conjunction with low tide, cooler sediment temperature was recorded across all sampling locations by comparison to average tropical weather. Environmental parameters for the sampling sites in January and February are summarised in Table 1. A PCA of environmental data shows most locations as distinct groups (Figure 3). For instance, samples in Pantai Jerejak were spread on the upper right corner, and samples in Tanjung Bungah were located on the upper left-hand side. The first two axes accounted for 65.8% of the total variance. Pantai Jerejak was mainly characterized with positive correlation by salinity and ammonia, while Teluk Bahang by phosphate, nitrite, and nitrate.

Similarly, light availability was also low due to the sun angle, ranging from 156.08±2.88 to 266.98 ± 36.07 µmol photons m^{-2} s^{-1} across sampling locations. Pantai Jerejak recorded the highest porewater salinity for both months (Average of site A and B, January: ~ 31.79 and February: ~ 31.00) and varied significantly ($p < 0.001$) between Teluk Bahang and Tanjung Bungah. The post-rainy condition caused a significant decrease in porewater salinity in February at all locations, with the lowest value recorded in Tanjung Bungah Site B (20.67 ± 1.15). A small stream in Tanjung Bungah and Teluk Bahang had caused a significant difference in salinity between sites A and B in both locations. Porewater pH ranged from 7.43 to 8.10 for all sampling sites. In January, the low temperature and salinity did not impact the pH, as no significant changes ($p = 0.907$) were observed during both months.

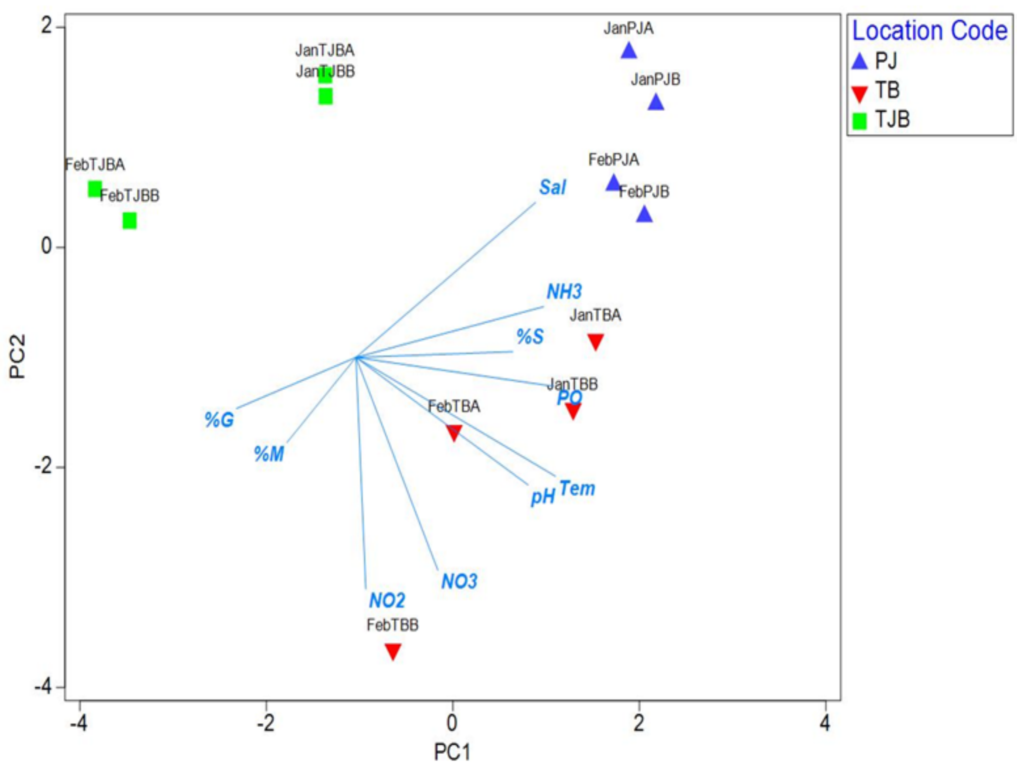


Figure 3. Principal Components Ordination (PCA) of environmental variables. Vector plots indicate the direction and size of the correlation between PC axes and variables. *(Sal: Salinity, NO2: Nitrite, NO3: Nitrate, PO: Phosphate, NH3: Ammonia,Tem: Temperature, %S: Sand: %G: Gravel and %M: Mud)*

Table 1

Environmental parameters of the sampling sites on the Pulau Pinang coastal area (Malaysia)

Location		Variables			
		ST	Light	PWS	PWpH
PJ A	January	29.10 ± 0.40^{a}	214.10 ± 0.00^{a}	31.57 ± 0.49^{a}	7.76 ± 0.05^{a,b}
PJ B		29.87 ± 0.06^{a}	214.87 ± 1.29^{b}	32.00 ± 0.00^{a}	8.01 ± 0.01^{b,c}
TB A		29.00 ± 0.53^{a}	166.80 ± 19.79^{c}	25.00 ± 0.00^{a}	7.70 ± 0.13^{a}
TB B		29.53 ± 0.12^{a}	156.08 ± 2.88^{c}	24.77 ± 0.68^{a}	8.09 ± 0.10^{c}
TJB A		29.00 ± 0.01^{a}	230.08 ± 22.34^{d}	25.00 ± 0.00^{b}	7.27 ± 0.06^{a}
TJB B		29.00 ± 0.01^{a}	266.98 ± 36.07^{d}	24.67 ± 0.58^{b}	7.43 ± 0.01^{c}
PJ A	February	29.07 ± 0.64^{a}	253.68 ± 7.24^{a}	31.00 ± 1.00^{a,b}	7.72 ± 0.14^{a,b}
PJ B		29.77 ± 0.06^{a}	266.19 ± 12.47^{a}	31.00 ± 0.00^{a}	8.10 ± 0.10^{b}
TB A		29.07 ± 0.64^{b}	659.52 ± 29.23^{b}	23.03 ± 2.00^{b}	7.81 ± 0.18^{c}
TB B		29.77 ± 0.06^{b}	637.55 ± 3.86^{b}	20.93 ± 1.01^{b}	8.03 ± 0.34^{a,c}
TJB A		29.20 ± 0.26^{b}	543.75 ± 192.20^{a}	22.93 ± 2.06^{a,b}	7.48 ± 0.02^{a,c}
TJB B		29.90 ± 0.10^{b}	681.73 ± 29.54^{a}	20.67 ± 1.15^{a}	7.59 ± 0.02^{c}

Note: ST indicates sediment temperature; light expressed as µmol photosynthetic photon flux density (PPFD) m^{-2} s^{-1}; PWS indicates porewater salinity, and PWpH indicates porewater pH. Values are reported as mean ± SD. Same letter denotes no significant differences between different sites or months, and different letter denotes significant difference by p <0.05 as determined by ANOVA and subsequent Tukey's post hoc test.

Nutrient concentrations differ significantly (ANOVA, $p < 0.001$) between all sites (Figure 4). A progressive improvement in water quality was noticeable in the locations that were further from rapid developments and mariculture activities. Teluk Bahang which is located near the mariculture facilities had the highest orthophosphate (0.385 mg/L) and nitrate (0.310 mg/L) concentrations in January. However, in February, post-rainy conditions have caused a decrease in nitrate and ortho-phosphate concentrations across all stations. Ammonia concentrations, however, were not impacted and remained low at all locations. Pantai Jerejak, located near the industrial and residential areas, recorded high ammonia concentrations (average: 0.070 mg/L).

Taxonomic Composition and Abundance of Benthic Diatom

Light microscopy examination revealed a total of 53 species of diatoms representing 27 genera across the sampling locations. Diatom dominated the benthic microalgae at all locations, though other groups (Dinoflagellate) were present at lower densities. Thus, in this study, only diatoms were observed. The genera containing the highest number of species (number given in parentheses) were *Amphora* C.G Ehrenberg ex Kützing (5), *Cocconeis*

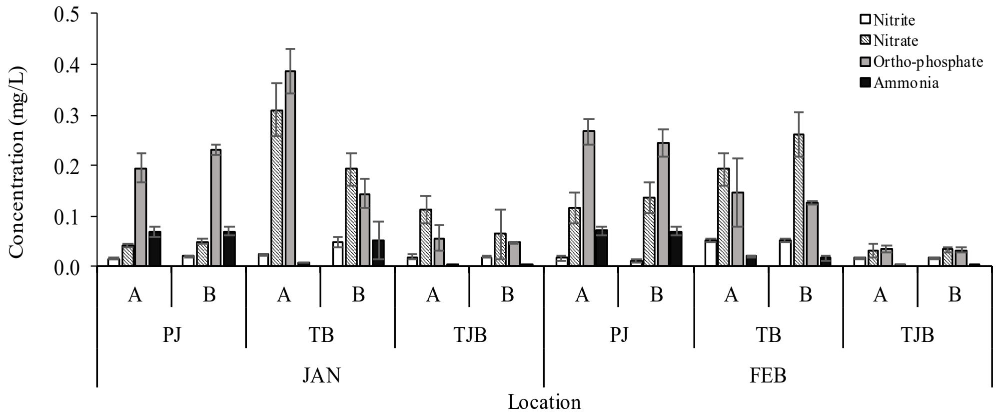


Figure 4. Porewater nutrients concentrations (mg/L) at the sampling sites on the Pulau Pinang coastal area

Ehrenberg, 1836 (6), and *Thalassionema* Grunow ex Mereschkowsky (5). Overall, the Genus *Cocconeis* was found to be the dominant genus for all locations and sites, whereby Pantai Jerejak (19.94%), Teluk Bahang (15.50%), and Tanjung Bungah (30.94%) (Figure 5). In terms of species dominance, Pantai Jerejak and Tanjung Bungah were dominated by *Cocconeis peltoides* and Teluk Bahang by *Navicula peregrina.*

The total abundance was significantly different ($p < 0.001$) among locations. Tanjung Bungah and Teluk Bahang have higher cell density by comparison to Pantai Jerejak. However, the total number of species and genera were relatively similar among locations (Table 2). The three diversity indices, species diversity (Shannon Index), evenness (Pielou Index), and richness (Margalef Index), used to differentiate both months and locations, are shown in Table 2. Due to the similarity in species composition, there were no significant differences ($p > 0.05$) between locations, sites, and sampling months. Shannon Index ranged between 2.93 to 3.12, Pielou Index was between 0.49–0.61 dan 6.72 to 5.95 for Margalef Index.

The n-MDS performed (two-dimension, stress = 0.1) on the relative abundance of 53 species collected at all locations and sites (12 samples) pointed out an apparent similarity among diatom communities of coastal intertidal areas of Pulau Pinang (Figure 6). Pantai Jerejak groups were easily distinguished from Tanjung Bungah and Teluk Bahang. The average dissimilarity between Pantai Jerejak and Teluk Bahang is 30.41%, Pantai Jerejak, and Tanjung Bungah 30.79%, Teluk Bahang and Tanjung Bungah 28.95% (SIMPER analysis).

Table 2

Species number, diversity, evenness, richness, abundance, and chlorophyll a biomass at three sampling locations during the study period

Sampling Location (Site)		Species Diversity (Shannon Index)	Species Evenness (Pielou Index)	Species Richness (Margalef Index)	Total Genera	Total Species	Abundance (cell x 10^3 cm^{-2})
PJ A	**JANUARY**	3.09 ± 0.13	0.61 ± 0.08	5.95 ± 0.13	23	39	361.04 ± 65.61
PJ B		3.02 ± 0.07	0.58 ± 0.08	6.24 ± 0.23	24	42	491.95 ± 66.92
TB A		3.00± 0.15	0.52 ± 0.08	6.26 ± 0.46	20	44	395.56 ± 35.83
TB B		3.08 ± 0.15	0.54 ± 0.05	6.30 ± 0.42	26	49	553.26 ± 32.33
TJB A		3.00 ± 0.17	0.52 ± 0.08	6.02 ± 0.61	24	46	524.59 ± 32.35
TJB B		3.09 ± 0.16	0.56 ± 0.10	6.22 ± 0.12	25	47	481.01 ± 111.95
PJ A	**FEBRUARY**	2.93 ± 0.10	0.49 ± 0.05	6.19 ± 0.51	22	41	416.31 ± 66.57
PJ B		3.20 ± 0.09	0.59 ± 0.00	6.72 ± 0.52	25	49	429.33 ± 54.47
TB A		3.16 ± 0.10	0.55 ± 0.02	6.66 ± 0.54	25	47	552.69 ± 60.82
TB B		3.25 ± 0.04	0.60 ± 0.04	6.60 ± 0.09	26	48	554.96 ± 59.38
TJB A		2.94 ± 0.13	0.47 ± 0.04	6.24 ± 0.42	24	47	549.68 ± 56.38
TJB B		3.08 ± 0.21	0.52 ± 0.11	6.54 ± 0.20	24	45	593.25 ± 116.71

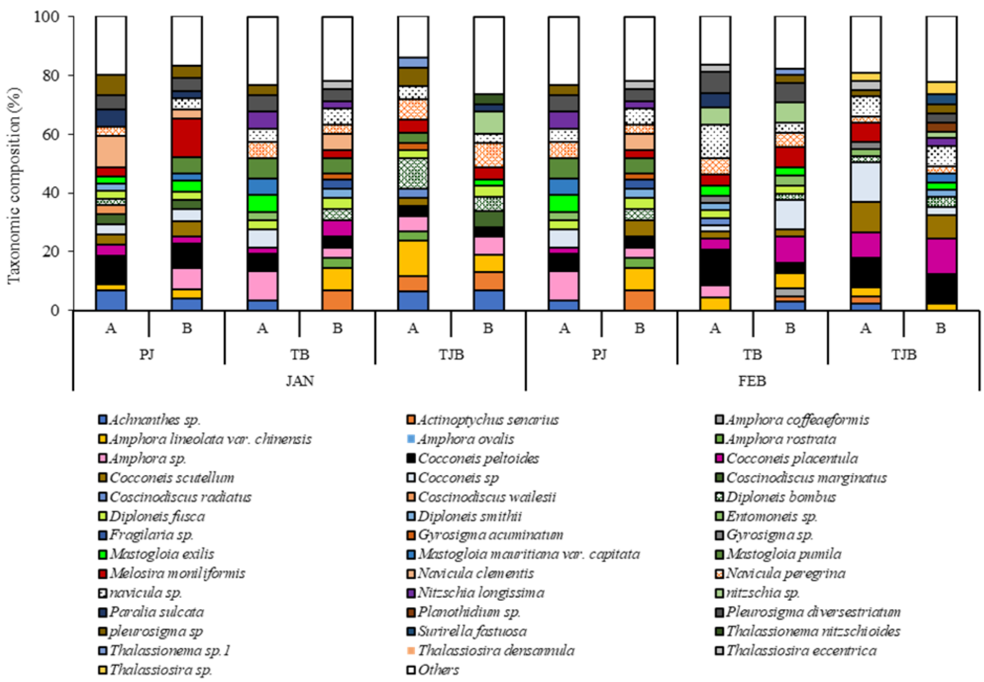


Figure 5. Taxonomic composition of benthic diatom communities of individual samples collected from three sampling locations. *(The species with relative abundance above 2% are presented, with the remaining species grouped as "others'. The relative abundance is presented as the percentage of the total cells counted)*

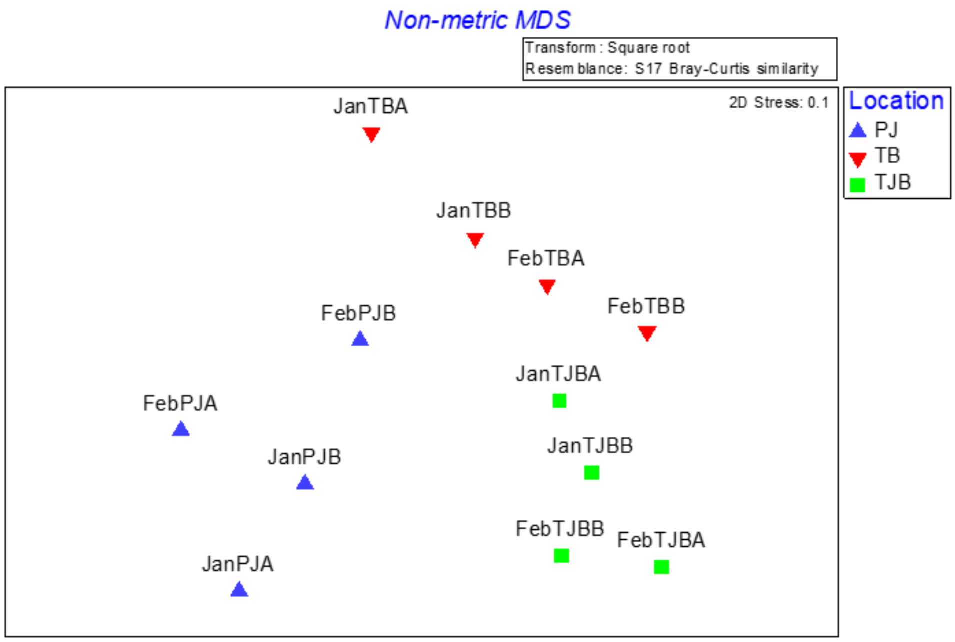
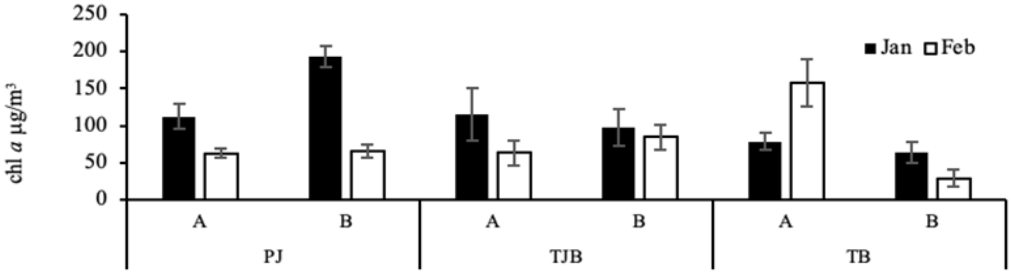


Figure 6. Classification of samples based on species composition by non-metric multidimensional scaling (nMDS)

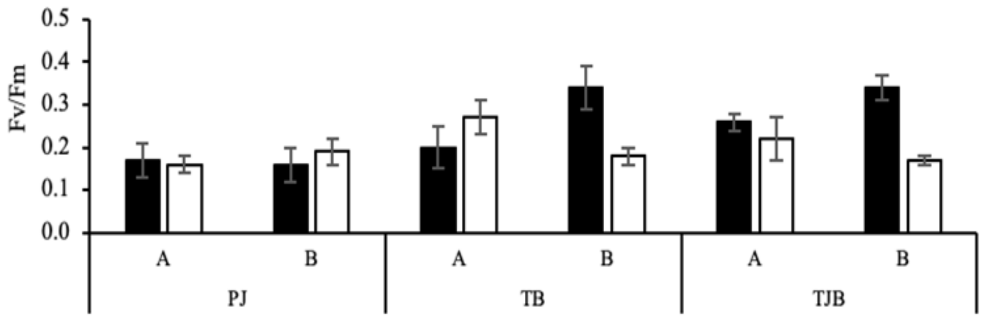
Benthic Chlorophyll *a* Biomass and Photophysiological Parameters

There was no significant difference ($p = 0.476$) between all sampling locations in benthic chlorophyll *a* concentration. However, significant differences ($p < 0.05$) were observed within the sampling site at each location. The highest value of 193.23 ± 14.36 µg/m^3 was recorded at Pantai Jerejak, Site A (Jan) and the lowest value at Teluk Bahang, Site B (Feb) (Figure 7a). However, the post rainy condition and the sediment composition changes have caused a significant decrease ($p < 0.05$) in chlorophyll *a* biomass, mainly in Teluk Bahang (Figure 7a).

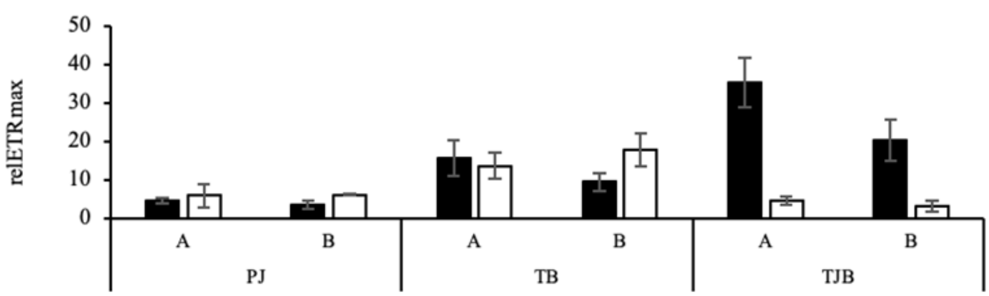
The maximum quantum yield recorded at all locations ranged from 0.170 to 0.340, which is relatively low (Figure 7b). In general, higher values were recorded in January ($p > 0.05$) compared to February. The highest F_v/F_m values were measured in Tanjung Bungah (0.34 ± 0.03) and Teluk Bahang (0.340 ± 0.05). In contrast, the lowest F_v/F_m values (~ 0.16 – 0.19) were measured at Site A (Pantai Jerejak) for both months were the highest in Tanjung Bunga (Site A (Jan): 35.51 ± 6.48 and B (Jan): 20.32 ± 5.26) but the value decreased to almost 80% in February. The rETRmax values ranged from 3.45 to 35.51 for all locations (Figure 7c). Similar patterns were observed for the photosynthetic efficiency values with significant differences between sampling time ($p = 0.035$) and locations ($p = 0.001$). The α values were the highest in Teluk Bahang and lowest in Pantai Jerejak, with significant differences between all sampling sites ($p < 0.05$) (Figure 7d). The mean E_k (light acclimation) parameter ranged between 67.96 ± 20.94 to 236.71 ± 50.39 µmol photons m^{-2} s^{-1}, with low values recorded in Teluk Bahang (Figure 7e).



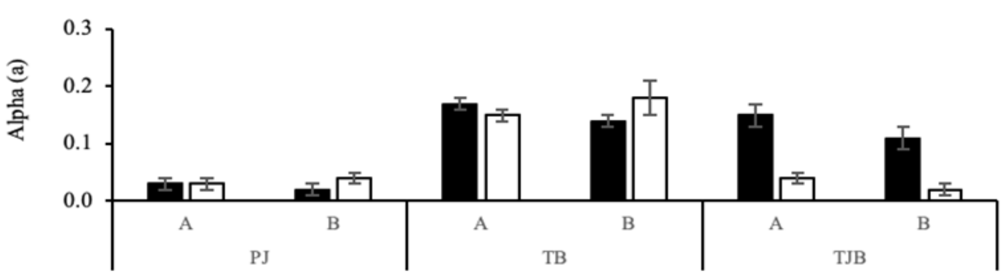
(a)



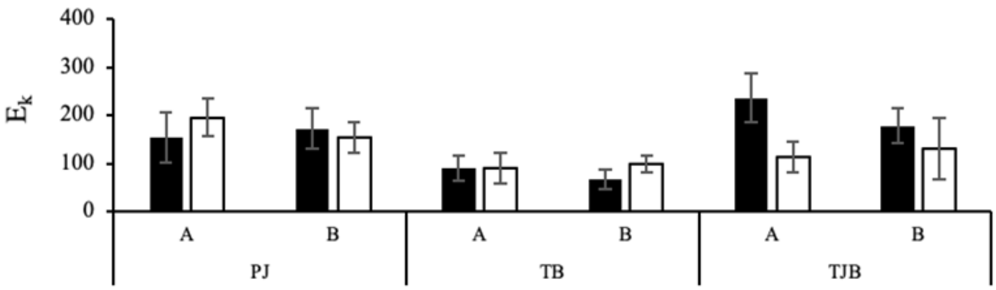
(b)



(c)



(d)



(e)

Figure 7. The measurement of a. Chl *a* biomass (μg/m^3) and photosynthetic parameters: b. Maximum quantum yield (F_v/F_m), c. relative electron transport rate (rETRmax), d. photosynthetic efficiency (α : μmol photons m^{-2} s^{-1}) and e. Photoacclimation Index (E_k: μmol photons m^{-2} s^{-1}) in the three sampling sites

DISCUSSION

Diatom Assemblages and Their Environmental Significance

Diatom is the dominant group of the intertidal microphytobenthos composition and has been noted as one of the most crucial elements in aquatic ecosystems. Despite their ecological role, very little is known about the distribution and ecology in tropical coastal intertidal flats, especially in Malaysia. The qualitative distribution of benthic diatom is one of the fundamental characteristics of intertidal flats. Like many other benthic diatom assemblages (Dalu et al., 2016), the composition and structure of benthic diatom inhabiting Pulau Pinang intertidal flats have resulted from a complex interaction of environmental variables, mainly nutrients, salinity, and temperature.

The genus *Amphora*, *Cocconeis,* and *Navicula* were noted as the dominant genus in this study, which is also prevalent in many intertidal flats, both in tropical and temperate (Du et al., 2009; Jordan et al., 2010; Salleh & McMinn, 2021). The benthic diatom inhabiting the Pulau Pinang coastal areas were distributed irregularly, and their species diversity is relatively low compared to other studies in the tropics (Chen et al., 2020). Similar low diversity was noted by McMinn et al. (2005) in the coastal areas of Pulau Pinang. Although having a similar number of species at all locations, the dominant genus/species varied between sites and months. The differences in the dominant genus/species at the stations each month can be explained by the differences in sediment nutrients, salinity, and *in-situ* temperature at each site. *Cocconeis peltoides* and *Navicula peregrina* were dominant in this study in most sampling sites. It is noted that diatom from both genera is dominant in many marine sediment surface (Mitbavkar & Anil, 2006). Genus *Cocconeis* was predominantly dominant in Tanjung Bungah, and *Navicula* was relatively evenly distributed in all locations.

The concentration of Chl *a* is generally used as an index of productivity (MacIntyre & Cullen, 1996). One of the main factors governing benthic diatom assemblages is substratum type (Cahoon et al., 1999) and is considered an essential variable in influencing benthic microalgae biomass and composition. For example, Jesus et al. (2009) noted higher diatom biomass in sandier substrates, while others contradict this finding and reported higher diatom biomass associated with muddier sediment (Du et al., 2009). Sandier substrates dominated sediment in Pantai Jerejak, and Tanjung Bungah noted higher chl *a* biomass than Teluk Bahang. Although studies have recorded correlations between chl *a* biomass and pore water nutrients (Magni & Montani, 2006). Du et al. (2009), noted that this is due to large oscillations of nutrient concentration in intertidal flats. However, in our study, chl *a* biomass was mainly influenced by salinity. Pantai Jerejak, with the highest chl *a* biomass, also recorded the most elevated salinity. The runoff from a small stream in Tanjung Bungah and Teluk Bahang has a significant influence on the diatom composition. Salinity is also one of the main parameters attributed to the diatom diversity and acts as a limiting factor influencing the community's distribution (Häusler et al., 2014).

Similarly, Darif et al. (2016) noted a considerable variation in salinity within the spatial scale (min value: 15.0 and max value 30.3) in Tanjung Bungah, which has impacted the presence of the macrobenthos. Generally, changes in the salinity of the intertidal habitats are due to the influx of freshwater from land runoff; caused by monsoon or tidal variations. Various studies have highlighted the importance of salinity (De Troch et al., 2012) gradients in marine ecosystems contributing to the spatial variability in these systems' biology (Thornton et al., 2002). Salinity and temperature were the most strongly related variables to assemblages' composition in intertidal areas. Thus, it is an essential factor in controlling diatom distribution within the coastal ecosystem in Pulau Pinang. Regardless of the many studies on the relationship between environmental variables and diatom assemblages, determining the primary regulating variables at most coastal sites is still complicated. Main environmental variables are usually interrelated with other organism distribution in coastal areas. Therefore, further studies and frequent monitoring are needed to highlight the critical relationship between biofilm taxonomic composition and water chemistry to identify species' ecological preferences.

***In-situ* Photophysiological Responses During Low Tide Exposure**

Maximum quantum yield (F_v/F_m) values can be used as a sensitive indicator of photosynthetic stress (Vieira et al., 2016). The F_v/F_m values recorded during low tide at all sites were relatively low compared to values of 0.650 for healthy microalgae (Schreiber, 2004). Although exposed to lower light intensity, F_v/F_m values in Pantai Jerejak were always low. F_v/F_m values indicated that benthic diatom in Teluk Bahang and Tanjung Bungah were relatively healthier or less impacted by environmental stressors (e.g., nutrient limitation, harsh temperature, or high dissolved oxygen concentration) (Li et al., 2021). Also, F_v/F_m was also observed in samples with high mud content. Further analysis and prolong monitoring is needed to determine whether this is caused by grain size (shading) as vertical migration activities might be less possible as 10 s intervals in RLCs were used here, thus minimising the confounding effects of vertical cell migration (Perkins et al., 2010b; Serôdio et al., 2006).

RLCs characterised the *in-situ* physiological response to temperature and salinity changes during low tide exposure. The parameters generally declined, corroborating those changes in temperature and salinity resulted in a decrease in photosynthetic activity. However, there is no evidence of photoinhibition, possibly due to the downward migration or the presence of photoprotective pigments (Jesus et al., 2006). rETRmax is a function of the enzymatic processes that depend on temperature, nutrient availability, light history, biochemical composition, and species composition, among other factors (e.g: Domingues et al. 2012; Laviale et al. 2015; Li et al. 2021). In this study, rETRmax was relatively lower than other tropical areas. Similarly, low values were also recorded by McMinn et al. (2005) during their study in the coastal area of Pulau Pinang. Adding to this, as we used the Pocket-PAM, this could explain the variations. Thus, the values reported in this study are only relative.

Lower E_k values were noted for muddier sediment in Tanjung Bungah, supporting prior studies that diatom communities inhabiting muddier sediment exhibit lower light acclimation than sandier sediments (Cartaxana et al., 2016). It is noted that a well-acclimated community would be expected to have an E_k value similar to the *in-situ* PAR as they can adjust their metabolism to maximise their response to light (Serôdio et al., 2005). The photosynthetic efficiency (α) was relatively low in Pantai Jerejak, whereby *in-situ* PAR was always low in both sampling months. It is expected that low light acclimated communities would have a high α value as they are more effective at rapidly utilising light at low irradiances (Ralph & Gademann, 2005) as observed in Tanjung Bungah. Hence, this indicates that benthic diatom communities in Pantai Jerejak were severely adapted to the ambient PAR and light limitations. However, a more extended monitoring period would be needed to determine these communities' ability to adapt to their light environment. The highly variable environmental conditions in the intertidal flat are likely to cause damage to the photosynthetic apparatus, mainly through reactive oxygen accumulation (Goss & Lepetit, 2015).

CONCLUSION

In summary, this study represents a snapshot of benthic diatom communities in three coastal intertidal areas in Pulau Pinang, whereby 53 taxa were observed. The obtained results represent the photosynthetic adaptation of benthic diatom to the changes in environmental factors, mainly salinity and temperature, while the constant low light did not significantly impact benthic diatom. The decrease in salinity resulted in lower photosynthetic capacity but did not induce photoinhibition. Our study lacks adequate long-term data to indicate specific environmental variables that significantly affect intertidal distributions of diatoms and their photosynthetic responses. However, the findings from this study provide baseline knowledge of diatom composition, photosynthetic health, and the relationship between their abundance and variations of environmental variables in the intertidal flats.

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