SPECIES COMPOSITION AND SPATIAL VARIATION OF BENTHIC DIATOMS AT INTERTIDAL ZONE UNDER THE INFLUENCE OF ENVIRONMENTAL PARAMETERS

by

NOOR ALIA BINTI AHMAD NORDIN

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"Have you thanked a diatom today?"

- Joshua Stepanek

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C°	degree Celsius
ppt	parts per thousand
µg/g DW	microgram per gram of dry weight
$\mu mol\ m^{-2}\ s^{-1}$	micro mole per meter squared per second
$cell/m^2$	cell per meter squared
NO_3^-	nitrate
NO_2^-	nitrite
NH_3	ammonium
PO_4^{2-}	ortho-phosphate
$F/F_{m'}$	effective quantum yield
F_v/F_m	maximum quantum yield
r	Pearson's Correlation Coefficient
ANOVA	Analysis of Variance
CCA	Canonical Correspondence Analysis
PAST	Paleontological Statistics Software Package for Education and Data Analysis
ICP-OES	Inductively Coupled Plasma – Optimal Emission Spectrometry
ISQGs	Interim Marine Sediment Quality Guidelines
SEM	Scanning Electron Microscope
SPSS	Statistical Package for Social Science
SRM	Standard Reference Material

UPW	Ultra-Pure Water
ACHN	*Achnanthes*
ACTCY	*Actinocyclus*
ACTNP	*Actinoptychus*
AMP	*Amphora*
BAC	*Bacillaria*
BIRE	*Biremis*
COSCI	*Coscinodiscus*
CYCL	*Cyclotella*
DELP	*Delphineis*
COCCO	*Cocconeis*
CERA	*Ceratoneis*
CYLND	*Cylindrotheca*
DIPLO	*Diploneis*
FRUS	*Frustulia*
FRA	*Fragilaria*
GRAM	*Grammatophora*
GYRO	*Gyrosigma*
LYR	*Lyrella*
MAST	*Mastogloia*
NAVI	*Navicula*
NITZ	*Nitzschia*

THALN	*Thalassionema*
ODON	*Odontella*
PLAN	*Planothidium*
PARA	*Paralia*
STAU	*Staurosirella*
PLEU	*Pleurosigma*
PINN	*Pinnularia*
SYND	*Synedra*
THALS	*Thalassiosira*
CAMP	*Campylodiscus*
TRACH	*Trachyneis*
RHOP	*Rhopalodia*
SURRI	*Surirella*

KOMPOSISI SPESIS DAN VARIASI RUANG DIATOM BENTIK DI KAWASAN AIR PASANG SURUT DI BAWAH PENGARUH PARAMETER ALAM SEKITAR

ABSTRAK

Kajian ini bertujuan menentukan taburan ruang diatom bentik di kawasan air pasang surut yang berlainan faktor alam sekitar. Perubahan ruang diatom bentik dibawah pengaruh pemboleh ubah alam sekitar turut dikaji. Analisis Korespondensi Kanonik (CCA) telah dijalankan bagi mengenalpasti hubungan antara faktor alam sekitar dan komuniti diatom. Sampel sedimen diambil semasa air surut di enam stesen persampelan sebanyak empat kali, iaitu pada bulan November 2013, Februari 2014, Julai 2014 dan Disember 2014 di kawasan persisiran pantai Tanjung Bungah, Pulau Pinang, manakala pada bulan Ogos 2014, Januari 2015, Mei 2015 dan September 2015 di kawasan muara sungai Tanjung Rhu, Langkawi. Tanjung Bungah adalah kawasan persisiran pantai dengan sedimen lumpur pasir berbatu campuran komposisi pasir yang tinggi (46%), dan batu (35%), manakala Tanjung Rhu pula merupakan muara sungai dengan sedimen berbatu pasir, yang terdiri daripada lumpur (12%), pasir (61%) and batu (22%). Sejumlah 51 spesis (Tanjung Bungah) dan 56 spesis diatom (Tanjung Rhu) telah dijumpai dalam kajian ini. Stesen berpasir di kedua-dua kawasan kajian didapati mempunyai jumlah diatom dan komposisi spesis pada perkadaran yang lebih tinggi daripada tapak yang berlumpur, dimana kedua-dua kawasan ini didominasi oleh diatom epipsammik. Kawasan persisiran pantai Tanjung Bungah telah didominasi oleh *Navicula* dan *Thalassiosira*, manakala kawasan muara sungai Tanjung Rhu oleh *Amphora* dan *Cocconeis*. Tanjung Rhu mempunyai kepelbagaian diatom tinggi (H' = 1.209), manakala Tanjung Bungah secara relatif mempunyai kepelbagaian diatom

rendah (H' = 1.097). Tambahan pula, perubahan dalam komposisi sediment telah menjejaskan variasi ruang komuniti epipelik dan epipsammik dalam kajian ini, dimana dominasi genus diatom yang berbeza ditunjukkan dikedua-dua kawasan. Ordinasi statistik CCA menunjukkan bahawa nutrien terutamanya menunjukkan hubungan yang kuat terhadap taburan kelimpahan diatom di kedua-dua kawasan. NH_3 dan kemasinan telah menjejaskan taburan komuniti diatom di Tanjung Bungah (35.1%), manakala NO_3^- dan lumpur dilihat mempunyai hubungan yang kuat dengan komuniti diatom di Tanjung Rhu (70%). Walaubagaimanapun, turun naik kemasinan telah diperhatikan di kedua-dua kawasan air pasang surut yang menunjukkan kehadiran genera yang toleran terhadap kemasinan (*Thalassiosira* dan *Cocconeis*), selain daripada perhubungan nutrien, suhu dan biomas. Di Tanjung Bungah, *Thalassiosira* mendominasi Wet 2 di stesen dengan kemasinan yang rendah (A dan F) dan suhu rendah (23.8, 24^{0}C) yang menunjukkan adaptasi diatom ini pada kandungan NH_3 yang tinggi, turun naik kemasinan dan perubahan keadaan yang melampau. Dalam pada itu, perkaitan yang kuat diantara kandungan NO_3^- dan PO_4^{3-} di Tanjung Rhu, berdasarkan ordinasi statistik CCA membenarkan perkembangan *Cocconeis*, selain keadaan kemasinan yang rendah yang membolehkan genus ini toleran terhadap perubahan kemasinan. Oleh itu, kajian ini menunjukkan variasi ruang diatom bentik dipengaruhi oleh nutrien, sementara peralihan dalam kemasinan mempengaruhi pemboleh ubah yang lain, seperti nutrien dan biomas. Walaubagaimanapun, perubahan komposisi sedimen di kedua-dua kawasan kajian turut menjejaskan taburan diatom epipelik dan epipsammik, selain daripada impak kuantiti logam berat terhadap populasi diatom.

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ABSTRACT

Benthic diatoms play a significant role in the primary productivity and sediment dynamics of coastal and estuarine intertidal ecosystems. Thus, this study aims to determine the spatial distribution of benthic diatoms in intertidal flats from different environmental gradients. The spatial changes of benthic diatom composition under the influence of environmental variables in the tidal flats were studied. Canonical Correspondence Analysis (CCA) was conducted to define the relationships between environmental variables and diatom communities. Sediment samples were collected during low tide at six sampling stations four times in November 2013, February 2014, July 2014 and December 2014 in the coastal area of Tanjung Bungah, and in August 2014, January 2015, May 2015 and September 2015 in Tanjung Rhu, Langkawi estuary. Tanjung Bungah is a coastal shore of muddy sandy gravel sediments containing of high sand (46%) and gravel (35%), while Tanjung Rhu is an estuary with sandy gravel sediments, comprising of mud (12%), sand (61%) and gravel (22%). In this study, a total of 51 species (Tanjung Bungah) and 56 species of diatoms (Tanjung Rhu) were found. The stations of greater sand in both intertidal areas had higher diversity and abundance of diatoms by comparison to the station with more mud, where Tanjung Bungah and Tanjung Rhu were dominated by epipsammic diatom assemblages. Tanjung Bungah coastal area was dominated by *Navicula* and *Thalasiosira*, while Tanjung Rhu estuary by *Amphora* and *Cocconeis*. Tanjung Rhu particularly showed higher diversity of diatoms (H' = 1.209), while Tanjung Bungah

had relatively lower diversity (*H'* = 1.097). In addition, sediment changes had affected the spatial variability of the epipelic and epipsammic assemblages in this study, where different dominance of diatom genus was shown in both sites. CCA ordinations revealed that, nutrients mainly showed strong relationships with the distribution of diatom abundances in both sites (TVE: 56 – 59%). NH_3 and salinity had affected the distributions of diatoms in Tanjung Bungah (35.1%), while NO_3^- and mud had greater relationships with the diatom community in Tanjung Rhu (72%). However, salinity fluctuations observed in both intertidals showed the presence of salinity-tolerant genera (*Thalassiosira* and *Cocconeis*), apart from the associations of nutrients, light, temperature and biomass. In Tanjung Bungah, *Thalassiosira* dominating Wet 2 in low salinity stations (A and F) and low temperatures (23.8, 24°C) indicated the adaptation of this diatom under high NH_3 supply, salinity fluctuations and extreme conditions. This implies *Thalassiosira* as an indicator of high nutrient conditions. Meanwhile, strong associations of NO_3^- and PO_4^{3-} levels in Tanjung Rhu, based on the CCA ordinations allowed the growth of *Cocconeis*, besides low salinity conditions making the genus tolerant to salinity changes. This study suggested that the spatial variation of benthic diatoms was mainly influenced by nutrients, while changes in salinity will affect other variables such as nutrients and biomass. However, the changes in the sediment compositions in both sites may have affected the distributions of epipelic and epipsammic diatoms, apart from the impact of heavy metal levels on the diatom population.

CHAPTER 1

INTRODUCTION

The coastal region and estuaries are dynamic and highly productive environment, specifically in nutrient cycling and organic matter storage (Levinton, 1995; Mann, 2000). The intertidal areas on the other hand, are constantly changing systems influenced by the high-energy levels of tidal flats. This phenomenon explained the distribution of microstructure of deposition and erosion of sediments (Swart, 1983). The extreme environment of intertidal from low to high tidal areas produces different levels of productivity, based on the tidal elevation and shore slope (Gray, 1981). Variations in tide levels create unstable conditions where greater extreme changes in temperature, salinity, dissolved oxygen and water content might comprehend (Hayward, 1994). For instance, changes in tide levels expose the sediments to the surroundings, causing desiccation of various organisms and drying of sediments in warm conditions and salinity fluctuations during rain. Benthic diatoms serve an important role as primary producers in the tidal flats of shallow-water bodies (Admiraal, 1984; Miller *et al.*, 1996), besides providing the source of organic matter for other inhabitants in the ecosystem (Decho & Lopez, 1993; Kawamura *et al.*, 1998).

1.1 The ecology of benthic diatom

Microphytobenthos are microorganism assemblages (diatoms, euglenids, crysophyceans and dinoflagellates), cyanobacteria and other photosynthesizing bacteria that dominate the benthic habitat surfaces (MacIntyre *et al.*, 1996). Diatoms on the other hand, of class Baccilariophyceae from division Chrysophyta (Bold & Wynne, 1985) are a distinct component of the microphytobenthos community at intertidal areas (Mitbavkar & Anil, 2002). Diatoms have significant, often highly embellished, siliceous cell wall consisting of valves joined by a belt-like band called a girdle band. They are pigmented and photosynthetic, but some could adapt living in the dark (heterotrophic) with adequate amount of organic carbon (Round *et al*, 1990). They were distinguished based on the structure of the valves, related in ecological studies (Dixit *et al.*, 1992).

Benthic diatoms populated on different types of substrates such as rocks, plants and sand, with some taxa lived on the sediment, but are not firmly attached to it (McIntyre & Moore,1977). They usually live within the surface layers on the top 10 mm of the intertidal (McIntyre *et al.,* 1996) and their sizes ranged from 5 µm to 500 µm (Cupp, 1943). Attached species typically occur in areas with strong currents, or harsh environments, while free-living diatoms are often found in sediment suspensions and burials (Round, 1971). Their habitats are scattered from salt marshes, submerged aquatic vegetation beds to intertidal and subtidal sediments including beaches.

In both estuarine and shallow coastal environments, diatoms are an integral and often dominant part of the benthic microalgal assemblages (Round, 1971; McIntire & Moore, 1977; Admiraal, 1984; Sullivan, 1999). Marine benthic diatoms living in the intertidal environment consists predominantly of species persistent to desiccation. Species of *Achnanthes, Cocconeis, Stauroneis, Toxonidea, Amphora* and *Catenula* are characteristic of sandy sediments within the epitidal zone (Round, 1971). The diatom taxa of silty sediments within the intertidal region can be very diverse, with Drum and Weber's record of 151 taxa (Round, 1971).

The composition and abundance of benthic diatoms are affected by various biotic (grazers and bioturbation) and abiotic (light intensity, sediment type, nutrient, salinity and hydrodynamic forces) factors. Benthic diatom constitutes the important components in all shallow-water ecosystems that provides adequate sunlight reaching the sediment surface and is required for primary production (MacIntyre *et al.,* 1996). These microorganisms also play an important part for nutrient and oxygen fluxes at the sediment water interface (Joergensen *et al.*, 1983; Asmus, 1984; Wiltshire *et al.*, 1996). They are used as environmental indicators due to their short life cycle which resulted in rapid community response to environmental changes (Dixit *et al.*, 1992; Sullivan, 1999).

Barranguet *et al.* (1997) mentioned that benthic diatom is usually found in abundance in the top 2 mm layer of the sediment surface especially in sediments with high organic content. They can be found mostly in sandy substrates down to 5 cm which contained a relatively lower organic content (MacIntyre *et al.*, 1996; Cahoon & Safi, 2002). Benthic diatoms act as a source of primary production and are able to change habitat characteristics (MacIntyre *et al.*, 1996). In addition, benthic diatom together with other aquatic photo-autotrophs can control their photosynthetic abilities in response to the disturbances in light, through physiological regulation and behaviour (Sêrodio *et al.*, 2006).

In addition, diatoms are ideal biological indicators for various types of pollution in aquatic systems (Lowe, 1974; Patrick & Palavage, 1994; Kelly *et al.*, 1995) and were being used in monitoring water quality assessments. They are recognized as the indicator of mud stability, pollution, diesel oil effect and heavy metal. It is important to grasp the contamination rate in benthic diatoms to detect any effect on the growth and species composition of benthic diatom communities, apart from taken into consideration of the increasing inputs of toxicants into the ecosystem (Stronkhorst *et al.*, 1994).

1.2 Intertidal coastal area

There have been limited studies on the distribution of benthic microalgae in tropical coastal areas, yet the scarce information suggests that this community is significant in various tropical coastal ecosystems (Kendrik *et al.*, 1998; Uthicke & Klumpp, 1998; Dizon & Yap, 1999). Benthic microalgal assemblages inhabiting tropical shores and shallow subtidal environments are exposed to increasing daily irradiances and temperatures, where both factors were shown to be affecting the activities of biofilms in temperate regions (Underwood & Kromkamp, 1999). In intertidal environments, biofilms will also be subjected to extreme salinities and dessication due to evaporation during tidal exposure. High light and temperature, under low nutrient levels will result in photoinhibition (Underwood, 2002). Thus, the ecology of such dynamic habitat in response to diatom populations should be highlighted and analyzed extensively.

1.3 Intertidal estuary

Meanwhile, estuarine ecosystems form transition zones between freshwater and marine bodies and are one of the most productive ecosystems in the world due to the nutrient inputs from both terrestrial and marine sources, as well as anthropogenic inputs (Anderson *et al.*, 2002; Bazin *et al.*, 2014). The mixing of marine and freshwater ecosystems and groundwater fluxes causes them to be characterized by the terms of physico-chemical variables (McLusky, 1993; Nodine & Gaiser, 2013), controlling the spatial distribution and composition of aquatic organisms in both systems (Nixon, 1995; Trigueros & Orive, 2000; Glibert *et al.*, 2006; Zhang *et al.*, 2009; Nodine & Gaiser, 2013; Bazin *et al.*, 2014;). Diatoms dominating the phytoplankton biofilms in the estuarine component are an important component, due to their high productivity rates (Brotas & Catarino, 1995; Underwood *et al.*, 1998), affect sediment changes (Paterson, 1994) and serve as carbon sources (Adam & Bate, 1999; Bate *et al.*, 2002; Carvalho *et al.*, 2002).

1.4 Objectives

The objectives of this research project are as follows:

1. To identify and determine the benthic diatom composition at the intertidal flats of Tanjung Bungah, Pulau Pinang and Tanjung Rhu, Langkawi.

2. To determine the spatial changes of benthic diatom composition under the influence of environmental variables in intertidal flats of Tanjung Bungah, Pulau Pinang and Tanjung Rhu, Langkawi.

1.5 Hypothesis

The hypotheses tested in this study were;

1. The species composition of benthic diatoms in both intertidal will vary in terms of abundance, dominance and diversity.

2. The spatial changes of benthic diatom composition in both intertidal will be affected by various environmental variables, chlorophyll *a* and quantum yield.

CHAPTER 2

LITERATURE REVIEW

2.1 Species composition of benthic diatom community

Diatoms are the main integral part of the microalgal biofilms which inhabit coastal and estuarine intertidal flats (MacIntyre *et al.* 1996; Underwood & Barnett, 2006). Benthic diatoms are the most abundant and most widely distributed group of microphytobenthos. It is considered to occupy the highest population densities and species richness in coastal habitats (Petrov *et al.*, 2010). According to Fourtanier & Kociolek (2003), more than 900 diatom genera are cosmopolitans, with some notable exceptions.

Benthic diatom communities inhabiting intertidal flats are categorized into two main groups, based on their structure and motility in sediments. The groups are epipelon and epipsammon (Round, 1971; Admiraal, 1984). Epipelic species are of free-living diatoms and can be found in abundance in mudflats, while epipsammic species are attached mainly to sand grains and therefore dominates the sandflats (McIntyre & Moorre, 1977; Hamels *et al.*, 1998). These sediment dwelling diatoms form a major component of benthic microalgae community in the intertidal region.

According to Sabbe (1997), epipelons are "all taxa that live in loose association with the sediment, including (mainly larger) motile pennate diatoms but also immotile taxa such as araphids and centrics". A broader definition of Sabbe (1997) explains the tychoplanktonic taxon accumulating in muddy sediments, such as *Cyclotella* or

Thalassiosira spp., apart from species residing in the interstitial environment within the sandy grains. On the other hand, epipsammons are "those taxon that live in close associations of either being attached or free-living with individual sediment particles" (Sabbe, 1997). This definition was portrayed in a slightly different perspective as compared to Round *et al.* (1990) or Admiraal (1984), who stated epipsammons as "attached or almost immobile diatoms growing on sand grains".

The ecology and taxonomy of benthic diatoms are significant than in the phytoplanktons as proposed by MacIntyre *et al.* (1996) and Miller *et al.* (1996). Based on Lewin & Lewin (1960), certain species from genus *Cyclotella*, *Navicula*, *Amphora*, *Podosira* and *Nitszchia* were able to live under no light source with organic materials. Dominant representatives of this microalgal community include several species of the genera *Amphora*, *Cocconeis*, *Diploneis*, *Navicula* and *Nitzschia* (Cahoon, 1999). Centric genera such as *Conscinodiscus*, *Skeletonema*, *Biddulphia* and *Thalassiosira* live within the sediment surfaces, compared to the pennate diatoms which were more diverse and abundant in the benthic habitat.

However, pennate diatoms are pervasive and often dominant in most studies, in terms of biomass (e.g. McIntire & Moore 1977, Admiraal 1984, Consalvey *et al.* 2004, and Underwood & Barnett, 2006). According to Mitbavkar & Anil (2002), most studies were more inclining towards epipelic and pennate diatoms. Other forms of diatom which reside, whether temporary or permanent, must not be left out. However, diatom populations can be better understood by analyzing and observing both forms equally.

There have been studies conducted in detail on the microalgae productivity (Musikasung *et al.* 2000) and microalgae taxonomy (Boonyapiwat, 1999) of Peninsula Malaysia. However, little is known for benthic diatom. In Malaysia, study on the diatoms in the Malaysia waters had been documented by Shamsudin (1990). Benthic studies on freshwater diatom have been carried out by Wan Maznah & Mansor (2002) in the Penang river basin, apart from a study done in Sarawak rivers (Hilalludin *et al.*, 2011). Limited studies were conducted on diatom inhabiting coastal environments.

The studies on microalgae in Malaysian coastal waters are limited specifically on benthic diatom species of marine ecosystem as more efforts have been focused towards riverine and estuarine diatoms. In Penang, studies on benthic diatoms by Cheng (2013) focused on the microphytobenthos (MPB) in Tanjung Bungah and Pantai Jerjak. Another study by McMinn *et al.* (2005) was conducted on benthic microalgae assemblages in Muka Head and nearby Songsong Islands, Penang. Therefore, more research on benthic diatom in marine waters should be actively implemented to ensure the contribution of these communities in the primary production of shallow waters (Underwood & Kromkamp, 1999).

2.2 Spatial variation of benthic diatom community

Ecological communities interacting in an area vary spatially and temporally (Andrew & Mapstone, 1987; Wiens, 1989; Barry & Dayton, 1991; Schneider, 1994, Horne & Schneider, 1995). Studies concerning the spatial distribution of organisms contribute towards a more comprehensive and better understanding of several limitations associated with community structure and function. Such information however, is best used to define different interspecific relationships, which to an extent determine community structure (Saburova *et al.*, 1995).

Spatial variations of communities are important in which, it helps to describe the ecological diversity of a population. Recognizing different relevant scales in both time and space is crucial to grasp the factors and processes creating patterns in biotic and abiotic components of ecosystems (Levin, 1992). There are wide ranges of applications to demonstrate diversity patterns. This include from identifying spatial barriers to recognize mechanisms that maintain biodiversity, to detecting environmental changes locally and regionally on various structure levels (Zvuloni *et al.*, 2010). In marine ecosystems, while regional diversity gives some insight into local patterns (Karlson *et al.*, 2004), local diversity may vary over those found on broader scales. For example, at broad scales, different processes may occur such as emigration, regional-scale speciation and gene flow, all of which associated with climate change and population interaction, apart from immigration which depends on hydrological pattern in the ocean (Veron, 1995).

Studies had shown that the benthic diatom communities are an extensive spatial structure and has a characteristic distribution of its own (Saburova *et al.*, 1995).

Patchiness was caused by massive growth of diatoms, dinoflagellates, and green and euglenoid algae has have been recorded by various researchers (Faure-Fremiet, 1951; Hirano, 1976; Fenchel, 1969; Joseph & Joseph, 1977; Blanchard, 1990; Paterson & Underwood, 1990).

Environmental variables such as light, salinity, nutrients, grazing and sediment type are responsible for the spatial variation and composition of benthic microalgae assemblages (e.g. Van der Grinten *et al.*, 2004; Jesus *et al.*, 2005; Van der Grinten *et al*, 2005;). According to Brotas *et al.* (1995), the spatio-temporal distribution of benthic microalgae biomass is partly restricted by tidal exposure and sediment properties. Inevitably, sediment type has been depicted as the variable which had affected the most to the variation of benthic microalgae communities (such as Cahoon, 1999; Honeywill *et al.*, 2002; Perkins *et al.*, 2003) apart from the significance of tidal height. According to Underwood & Barnett (2006), sediment types influence the inhabitance of benthic microalgae groups, whereby muddy grains were dominated by diatoms while the sandy sediments were populated with cyanobacteria and euglenids.

The spatial structure of benthic diatom communities in soft sediments has been studied for years, providing complicated spatial structure and aggregated characters of benthic microalgae communities (Saburova *et al.*, 1995). Majority of the previous works emphasized on the spatial community distribution and environmental factors contributing to diatom assemblages performed in temperate regions (Munn *et al.*, 2002; Potapova & Charles, 2002; Soininen, 2004; Cunningham *et al.*, 2005). However, basic knowledge of the diatom distribution patterns at different spatial scales is still very poor, especially in tropical coastal areas and estuaries.

Various studies on the ecology and primary productivity of marine benthic microalgae have been conducted in the tropical areas of Peninsular Malaysia (Mcminn *et al.*, 2005; Salleh, 2011; Cheng, 2013). However, further studies of the benthic diatoms based on different spatial scales have not been implemented in these tropical areas specifically. According to Bendetti-Cecchi (2001), spatial pattern analyses were common in population and environmental studies, but not many examined whether these patterns were homogenous at various scales. Characterising the spatial dynamics of the diatom assemblage structure to evaluate and predict changes in the ecosystem is important to understand the coastal and estuarine systems and community ecology. A spatial perspective of the community ecologically, helps in assessing the anthropogenic activities affecting the ecosystem (Craig & Bosman, 2013) apart from the various natural environmental factors influencing the community distribution.

Plante *et al.* (1986) had established three scales of heterogeneity in the distribution of chlorophyll *a* in sediments of the sandy sublittoral zone of the northern Mediteranean, namely microscale (3-10 cm), mesoscale (ca 10 m) and macroscale (ca 10 km). Chlorophyll *a* distribution in sediments was classified for three types of topographic features of the sediment surface, consisting of sandy ripple and two types of sandy waves. Therefore, chlorophyll *a* classifications are comparable in size, whereby at microscales (centimeters), the patchiness exhibited by the benthic diatoms is typically contributed to the interspecies relationships (Saburova *et al.*, 1995; Murphy & Tolhurst *et al.*, 2008). Variations in the spatial scales (centimetres to tens of meters) were found in relation with the interactions between sediment, microtopography, and diatoms (Plante *et al.*, 1986; Saburova *et al.*, 1995). In addition, various studies on spatial and seasonal distribution of benthic diatom assemblages in coastal and estuarine intertidal areas were conducted across the world (Round, 1971; McIntire & Moore, 1977) which was mainly in Northern European and North American estuaries.

Some of the factors contributing to the spatial distribution of diatoms include light intensity, hydrodynamics, and sediment grain size compositions (Fenchel, 1969; Coljin & Dijkema, 1981; Delgado, 1989). Changes in sediment properties have always been considered as the main environmental variable responsible for a rapid spatial disruption in tidal flat assemblages as mentioned in the works of Sabbe and Vyverman (1991) for instance, which affects the diatom community. According to Underwood & Kromkamp (1999), sediment is a major factor influencing the diatom abundance. Sediment composition does not only depend on hydrodynamic forces, but it is also closely related with other factors such as re-suspension, interstitial space, nutrient composition and irradiance (Cahoon & Safi, 2002).

Spatial distribution patterns of benthic microalgae species have been related to salinity, nutrient gradients, or tidal elevations (Ribeiro *et al.*, 2013). According to Admiraal (1984), most estuarine epipelic diatoms are various in their distribution and are able to comprehend wide ranges of environmental conditions. Accumulation of nutrients in the sediment produces higher benthic microalgae biomass in muddy substrates, as nutrients supported the growth of benthic diatoms (Cahoon & Safi, 2002). On the contrary, Mitbavkar & Anil (2002) found that the pennate diatom that lives in sediments did not show any significant effects on nutrient levels, but they discovered that nitrate and phosphate influenced the abundance of centric diatoms.

2.3 Influences of environmental variables on benthic diatom distribution

The distribution of benthic diatom biomass is influenced by tidal position, nutrient access and sediment properties (Guarini *et al.*, 1998; Light & Beardall 1998; Jesus *et al.*, 2009; Grinham *et al.*, 2011) as well as biological interactions with macrofauna (Pinckney & Sandulli, 1990) and other microorganisms (Danovaro *et al.*, 2001). These components affect greatly at various scales producing distinct patterns in terms of spatial forms in benthic diatom (Saburova *et al.*, 1995).

Environmental variables may change rapidly in intertidal flats (Admiraal, 1984; Paterson & Hagerthey, 2001) that diminish the photosynthethic productivity of benthic diatom (Blanchard *et al.*, 2004; Serodio *et al.*, 2008). To overcome the situation, they have adopted distinct behavioural and physiological responses, where only free-living diatoms residing in mudflats (epipelon) will be able to move out of the harsh environment at the sediment surface by migrating downwards towards the most ideal condition. Apart from that, other major factors governing the benthic diatom communities are light, temperature and nutrients (Allen, 1971). Limitations of these variables may have diverse effects on the growth rate, productivity anz acitivities of the benthic diatom community. Other environmental variables include salinity, sediment grain size and quantum yield.

2.3.1 Physical parameters

Changes in salinity has been less studied in benthic diatoms, as opposed to planktonic diatoms where it is known to cause changes in the diversity of species community as well as growth and photosynthesis (Thessen *et al.*, 2005; Dijkman & Kromkamp, 2006; Muylaert *et al.*, 2009; Petrou *et al.*, 2011). Salinity often coincides with other environmental variables such as light and temperature. As such, in high salinities, porewater evaporates in the upper-layer of the sediment, while nutrient levels were affected in low salinities condition, due to the release of estuarine rivers into the sea and estuary (Admiraal & Peletier, 1980; Thornton *et al.*, 2002; Underwood & Provot, 2000). Salinity fluctuations coupled with high light often impacts the diatom's growth, which occurs at salinity of 40 ppt and above (Natana Murugaraj & Jeyachandran 2007; Scholz & Liebezeit, 2012). This fluctuation causes the decline in the photosynthetic ability through photo-oxidative stress (Rijstenbil, 2003; Roncarati *et al.*, 2008) besides changing the motility of epipelic diatoms (Sauer *et al.*, 2002) in the sediments with the discharge of exopolysaccaharides (Apoya-Horton *et al.*, 2006).

Benthic diatom yields higher primary production in higher salinity conditions as mentioned by Blasutto *et al.* (2005). They reported that benthic diatom communities were more susceptible towards higher productivity in higher salinity, probably due to the production of more stable and less stress environment for benthic diatoms. However, in the euryhaline phytoplankton production, they found that salinity was not a limiting factor. According to Underwood *et al.* (1998), *Navicula gregaria* was found to be growing in oligohaline region and *Navicula phyllepta* in mesohaline, whilst *Pleurosigma angullatum* and *Pleurosigma vitrea* were found in polyhaline of Colne estuary, United Kingdom. In addition, the motility response of epipelic diatoms was suggested to accommodate the fluctuations in environmental conditions such as light and salinity at the surface of sediments (Admiraal, 1984).

Apart from salinity, pH is another environmental factor that should not be left out when evaluating the changes in the diatom distributions. According to Scholz & Liebezeit (2014), the differences in pH ranging from 6.5 to 8.5 had insignificant effects on the growth of benthic diatoms. Apart from that, different ranges of pH that determines which species is tolerant based on different alkalinities and acidities can affect the distribution pattern of benthic diatom species. The distributions of several species of *Eunotia* and *Neidium*, which are considered acidophilic taxa, are common in south-easthern river that often have low pH (Potapova & Charles, 2002). Duong *et al.* (2006) revealed the presence of planktonic and alkali-tolerant taxa in the Nhue river, Vietnam which indicated a moderate pollution. This can suggest the importance of pH ranges affecting the ecological preferences of diatoms and its relation to pollution.

2.3.2 Sediment composition

According to Mitbavkar & Anil (2006), sediment grain size fractions vary with different tidal zones and acts as predictors of some diatom genera, such as *Grammatophora, Coscinodiscus, Biddulphia, Bellerochea, Navicula, Cyclotella, Thalassiothrix, Raphoneis, Streptotheca* and *Pleurosigma*. The abundance of *Amphora* and *Grammatophora* were observed at 250 µm grain size fraction at 0 – 5 cm depth. At low tide, the diatom community was exposed to harsh environmental conditions. Dessication is detected as the major effect, since this zone is exposed to direct sunlight for longer hours compared to high tide. The sand becomes dry for longer periods and at greater depth, which influences the presence of bigger grain size in the low intertidal zone. Thus, the drying effect of this zone causes low community of benthic diatom (Bush, 1966; McIntire & Wulff, 1969). Furthermore, increasing chlorophyll *a* content was found in finer sediment particles (< 125 μm) as suggested by Cahoon (1999). They found that there was insignificant correlation between the fraction of fine grain sizes and benthic biomass, probably due to the increasing uptake of anthropogenic loadings of fine grain sizes. Consequently, this will decrease the productivity of marine ecosystems.

2.3.3 Nutrients

The species composition of benthic diatoms corresponds to sediment nutrient concentrations (Peletier, 1996; Underwood *et al.*, 1998; Sullivan, 1999; Underwood & Provot, 2000). This corresponds to study by Underwood (2002), who founds that the patterns of species abundance had shown nutrient preferences. Apart from that, increase in the sediment nutrient levels produces higher benthic diatom biomass in muddy substrates, as nutrients supported the growth of benthic diatoms (Cahoon & Safi, 2002). In the contrary, Mitbavkar & Anil (2006) found that nutrients did not play an important role for pennates which was found mainly in the sediments. However, they discovered that nitrate and phosphate had influenced the abundance of centric diatoms. The upwelling of surface waters and land runoffs cause the release of nutrients from sediments into the water columns, increasing nutrients for the centric diatoms to grow.

Philippart *et al.* (2000) found that under eutrophic and nitrogen-controlled conditions, a disparity increase had occurred in the abundance of large diatom species (individual cell size of 1000 μm^3). Moreover, they suggested that the increase in the phytoplankton biomass had enhanced benthic denitrification, which explained the nitrogen loss rates. However, Blackford (2002) stated that, nutrient stress is not a major limiting factor of benthic diatom communities because of the presence of benthic nutrient pools. The benthic diatom significantly affects the benthic trophic dynamics and biological processes despite not being significant towards nutrient uptake and cycling at regional inputs.

2.3.4 Heavy metal concentrations

Heavy metal contamination may influence the growth and physiological state of benthic microalgae communities. According to Peres *et al.* (1997) and Gold *et al.* (2002), they reported some changes in the structure of biofilms when exposed to metal contaminated sites. Diatom populations were discovered to be dissimilar in contrast to the polluted sites (Ivorra, 2000). Cell abnormalities altering the general cell shape or valve ornamentations were recognized under the influence of metal pollution. They were unlikely to be an indicator of such pollution (Dickman, 1998, Torres *et al.*, 2000; Gomez & Licursi, 2003).

Increasing toxicity of metals hinder the photosynthesis process, which in turn decreases photosynthetic availability (Rijstenbil *et al.*, 1994). A lower growth rate, apart from the ceasing of cell division and impaired diatom frustule were some of the impacts from anthropogenic contamination on benthic diatoms (Dickman, 1998). In community level, however, there had been evidences of reduced species diversity and richness due to the alteration of species composition and structural changes (Crossey & La Point, 1998). Although there were quite a few documentations on the metal contamination in lakes, the studies on the marine diatom was scanty regarding on anthropogenic pollution (Sullivan, 1999). This proved that more researches are needed to clearly define deleterious impacts of metal pollution towards benthic diatom community.

Several studies on heavy metal pollution have been conducted in Malaysia, such as Kamaruzzaman *et al.* (2008) and Mohd Zahir *et al.* (2012) in Langkawi, while a study of heavy metal contamination in Port Klang (Tavakoly Sany *et al.*, 2013) and Al Shami *et al.* (2011) in Juru River, Penang. The studies have been done on the contamination of heavy metal on coastal and estuarine environments. However, no studies have been done on the contamination of heavy metal concentrations on benthic diatom community in Malaysia.

Despite that, in the temperate region, studies on the benthic diatom response towards metal concentrations have been conducted (Cunningham *et al.*, 2003; 2005). Studies in tropical countries focused on contamination in rivers community (Bere, 2010) as well as in polluted marine environment towards the benthic diatom (Petrov *et al.*, 2010). Inevitably, less focus has been channelled towards the metal contamination on marine diatom communities. The responses of estuarine and coastal diatom species to pollution remain undiscovered (Sullivan, 1999). According to Stronkhurst *et al.* (1994), contaminants would affect the growth rate and species composition of benthic diatom communities but, with side effects for higher ranking organisms in the food chain. The composition of benthic diatom communities on the other hand, is recognized as a good indicator of anthropogenic metal contamination.

This may be important in observing the solution for environmental strategies in Antarctica and as well as other places (Cunningham *et al.*, 2005).

2.4 Chlorophyll *a* biomass

Benthic microalgae are important primary producers in shallow aquatic systems (MacIntyre *et al.*, 1996; Miller *et al.*, 1996; Cahoon, 1999; Underwood & Krompkamp, 1999). Benthic microalgal primary production contributes significantly to coastal carbon budgets (Underwood & Kromkamp, 1999) and microalgal biofilm associations with sediment-water nutrient exchanges and processes (Nedwell *et al.*, 1999) besides stabilizing the sediments (Paterson & Black, 1999). de Sousa *et al.* (1998) revealed that, very low microphytobenthic chlorophyll *a* levels (10.4-39.7 μm^{-2}) have been reported in most tropical coastal environments. In the subtropical Langebaan lagoon, South Africa, benthic microalgae had contributed approximately 22% of total primary productivity, with higher biomass and production recorded in the muddy than in the sandy fraction of the estuary (Fielding *et al.*, 1988).

de Jonge (1985) reported that diatoms were dominant in muddy sand grains, rather than in the bare parts of the sandy grains. This is due to the role of muddy grains as a substrate for diatoms on the tidal flats. Primary production rates were found to be lower at larger-sized sediment grains than the finer grain-sized sediments (Cook *et al.*, 2004a). Sin *et al.* (2009) discovered that, there was no significant relationship between the sandy grains and benthic chlorophyll *a* which suggested that sediment grain size was not the major factor controlling the benthic microalgal biomass in the Kwangyang Bay, South Korea. Sediment compositions were known as one of the factor influencing the biomass and composition of benthic microalgae (Cahoon & Safi, 2002). However, there have been diverging opinions with some reporting higher biomass in sandier substrates such as in Cahoon *et al.*, (1999) and others recording higher biomass in finer sediments (e.g., Cahoon *et al.*, 1999). Therefore, this may imply the importance of the sediment characteristics which may influence the benthic chlorophyll *a* biomass concentration in diatoms.

2.5 Quantum yield

Quantum yield analysis describes the processes taking place within a photosynthetic organism which focuses on the chlorophyll *a* fluorescence. Govindjee (1995) stated its importance in providing information on primary energy conversion. In this study, the photosynthetic activity of benthic diatom is observed by using the Pulse Amplitude Modulated (PAM) fluorometry. The application of PAM flourometry enables rapid assessment of the physiological status of benthic diatom. Based on Serôdio *et al.* (2001), the minimal fluorescence in the dark showed that the changes in chlorophyll *a* can be detected after being exposed to a series of irradiances or temperature ranges, which are the characteristics usually found during field samplings.

Moreover, under suitable range of light and temperature, there is a positive correlation between chlorophyll *a* in the top 200 μm sediment surface and the F_o^{15} (minimum fluorescence yield after 15 minutes of dark adaptation). From the observation of benthic diatom microfilm, Serôdio *et al.* (2001) stated that F_o was found to be the least sensitive to temperature and irradiance fluctuations in comparison with other photosynthetic parameters (Fm, $F_{m'}$ and F'). F_o also showed the least amount of species-specific variation. Thus, the change in minimum fluorescence might be proportionate to the change in chlorophyll which in turn affects the photosynthetic parameters.

2.6 Benthic diatom as environmental indicator

McCormick & Cairns (1994) stated that the benthic diatom's position at the lowest level of the food chain enables diatom population to provide various data on the ecosystem's physiological state as opposed to the use of common animal indicators. Diatoms exhibit several characteristics which enabled them to be excellent environmental indicators (Dixit *et al.,* 1992). Their sensitivity towards environmental changes and deliberately short life cycle has eventually brought towards an accelerated community response to change (Sullivan, 1999; Wan Maznah & Mansor, 2002). The worldwide distribution of various diatom species has proven that the results from studies from other different countries are still relevant (Round, 1993). Comparable ecological studies have even been recorded between Australian species and their morphological equivalents from the northern hemisphere (Reid *et al.*, 1995; Vyverman *et al.*, 1995; Hodgson *et al.*, 2000).

There are several major constraints identified with the usage of diatoms as bioindicators. One of them is the relatively substantial number of taxa (Kelly *et al.*, 1995). Relatively high numbers of valves counted per sample, further increase the complexity. For example, at least 300 counts of diatom valves of each sample are used (Van Dam, 1982; Sullivan & Moncreiff, 1988), although counts of 400-500 of sample sizes are usually documented (Cooper, 1999). The restraints of large occurring taxa and sample size can be comprehended by statistically analyzing the data with multivariate functions, such as factorial analysis and canonical correspondence analysis (CCA) that enables the relative correlation of multiple environmental factors to be attributed (Cunningham, 2003).