THE TAXONOMICAL AND ECOLOGICAL STUDIES OF PHYTOPLANKTON AND BENTHIC MICROALGAE IN TELUK BAHANG, PENANG

AYSHA MARIAM BINTI MOHAMED JAVEED

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by

AYSHA MARIAM BINTI MOHAMED JAVEED

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Aysha Mariam

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOL AND ABBREVIATION

°C	degree Celcius
µg/L	microgram per litre
µm	Micrometer
mg/L	milligram per litre
mg/m^3	milligram per meter cube
nm	nanometer
mm	millimeter
cm	centimetre
ml	millimeter
mg	milligram
km	kilometer
ppt	parts per thousand
NO_3^-	nitrate
NO_2^-	nitrite
NH_3	ammonium
PO_4^{2-}	phosphate
SiO_2	Silica
rpm	revolutions per minute
r	Pearson's Correlation Coefficient
%	percentage
$cell/m^2$	cell per meter squared
ANOVA	Analysis of Variance
CCA	Canonical Correspondence Analysis
SEM	Scanning Electron Microscope

KAJIAN TAKSONOMI DAN EKOLOGI FITOPLANKTON DAN MIKROALGA BENTIK DI TELUK BAHANG, PULAU PINANG

ABSTRAK

Perubahan dalam komposisi air dan sedimen memainkan peranan penting dalam mengawal taburan dan komposisi fitoplankton dan mickroalga bentik. Kajian ini meneliti komposisi taksonomi dan meneroka variasi masa taburan alga bentik dan fitoplankton yang dipengaruhi oleh kualiti air dan faktor alam sekitar. Sampel alga bentik dan fitoplankton diambil setiap bulan semasa air surut di Teluk Bahang untuk tempoh 18 bulan. Sebanyak 11 ukuran *in situ* seperti pemboleh ubah fizikal (kemasinan, suhu, pH dan oksigen terlarut), biomas klorofil (air dan sedimen), nutrien yang terdapat di permukaan air (NO_2^-, NO_3^-, NH_3, PO_4^{3-}, and SiO_2). Bacillariophyta (100%) mendominasi kedua-dua stesen bentuk (Stesen A dan B) dan di Stesen C, Baccilariophyta mendominasi sebanyak 87.76% dan diikuti oleh Dinophyta sebanyak (12.24%). Species dominan *Navicula menisculus* memiliki perkadaran komposisi yang tertinggi di kedua-dua Stesen A (13.9%) dan Stesen B (30.6%). Manakala species dominan *Bellerochea horologicalis* (61.1%) memiliki kadar tertinggi di antara keseluruhan spesies fitoplankton di stesen C. Canonical Correspondence Analysis (CCA) telah digunakan untuk mengenal pasti hubungan diantara spesies alga bentik dan fioplankton yang dominan dan pemboleh ubah persekitaran. Keputusan CCA memaparkan silika dan phosphate mempunyai kesan yang tinggi terhadap komposisi *Navicula menisculus* di stesen A. Manakala di stesen B, silika, phosphate dan ammonia memainkan peranan terhadap komposisi *Navicula menisculus.* Dalam tempoh 18 bulan kajian, terdapat perubahan taburan dan komposisi diantara species dominan. Analisa statistic menunjukkan bahawa komposisi dan taburan fitoplankton dan mickroalga bentik dipengaruhi oleh factor fizikal dan nutrien. Hasil dari kajian ini melaporkan 59 species yang terdapat dikawasan bentik (stesen A dan B) dan kawasan perairan (stesen C) di Teluk Bahang. Spesis bentik microalga di kedua-dua stesen A dan B didominasi oleh kumpulan Bacillariophyta (100%). Manakala, fitoplankton di stesen C di dominasi oleh kumpulan Bacillariophyta (87.76%) dan Dinophyta (12.24%). Stesen A dan B didominasi oleh spesis yang sama daripada kategori diatom (*Navicula menisculus*), manakala di stesen C, spesis diatom *Bellerochea horologicalis* mendominasi. Spesis *Navicula menisculus* di stesen A, didapati mempunyai komposisi peratusan yang tertinggi (13.9%) dan daripada keseluruhan kumpulan diatom dan mendominasi pada musim kering. Spesis *Navicula menisculus* juga mendominasi stesen B pada peratusan 30.6% pada musim hujan. Di stesen C, *Bellerochea horologicalis* mendominasi (61.1%) pada bulan kering. Hasil daripada pemantauan selama 18 bulan mendapati terdapat peralihan spesis diantara spesis dominan di ketiga-tiga stesen. Canonical Correspondence Analysis (CCA) digunakan untuk mengenal pasti factor alam sekitar yang mempergaruhi peralihan spesis dominan. Keputusan CCA menunjukkan bahawa factor alam sekitar tidak mepunyai kesan yang ketara terhadap peralihan spesis dominan di ketiga-tiga stesen selama 18 bulan. Regression digunakan untuk membuktikan hasil keputusan CCA. Silica didapati tidak mempunyai kesan yang kuat terhadap peralihan spesis *Navicula menisculus* di stesen A (r^2=0.379). Manakala, di stesen B, ammonia juga didapati tidak memperaguhi peralihan spesis *Navicula menisculus* (r^2=0.203). Phophat juga tidak mempegaruhi peralihan *Bellerochea horologicalis* di stesen C (r^2=0.203). Kajian ini menunjukkan bahawa perubahan bentik microalga dan fitoplankton tidak dipengaruhi parameter alam sekitar.

THE TAXONOMICAL AND ECOLOGICAL STUDIES OF PHYTOPLANKTON AND BENTHIC MICROALGAE IN TELUK BAHANG, PENANG

ABSTRACT

Variations in the composition of waters and sediment play an important role in regulating the temporal distribution and abundance of phytoplankton and benthic microalgae. This study examined the taxonomic composition, and study the temporal variability of benthic microalgae and phytoplankton in relation to water quality and environmental parameters. The sample collections were carried out monthly during low tide along the intertidal zone at Teluk Bahang, Penang for 18 months. A total of 11 in situ measurements were studied such as physical variables (salinity, temperature, pH, and dissolved oxygen), chlorophyll *a* biomass (water and sediment), pore and surface water nutrients (NO_2^-, NO_3^-, NH_3, PO_4^{3-}, and SiO_2). In this study, a total of 59 species were found in benthic (station A and B) and subtidal area (station C) of Teluk Bahang. Benthic microalgae species at two benthic stations (Station A and B) were dominated by Baccillariophyta (100 %) and phytoplankton species (Station C) were dominated by Baccillariophyta (87.76%) and Dinophyta (12.24 %). Station A and B were dominated by same species of diatom which is *Navicula menisculus* and in Station C, *Bellerochea horologicalis* dominated the subtidal area. The species *Navicula menisculus* had the highest percentage of the overall diatom species composition in both station A during dry months (13.9%) and in station B (30.6%) during wet months. While in station C, the dominant species *Bellerochea horologicalis* had the highest percentage of the overall phytoplankton species composition (61.1%) during dry months. During this eighteen months of period of this study, temporal variations of the benthic microalgae and phytoplankton species exist between the dominant species. Canonical Correspondence Analysis (CCA) have been used to identify the relationships between the dominant species of phytoplankton and benthic microalgae with environmental variables. CCA revealed that environmental parameters does not have strong effect on transition of dominant species of benthic microalgae in all stations (station A, B, and C). This result is proved using linear regression where effect between *Navicula menisculus* transition and silica in station A is low (r^2=0.379). Transition of *Navicula menisculus* in station B also was not strongly affected by ammonia (r^2 = 0.203). In station C, distribution of *Bellerochae horologicalis* was not strongly effected by phosphate (r^2 = 0.380). This study suggest that the temporal variation of benthic microalgae and phytoplankton was not strongly influenced by environmental parameters.

CHAPTER 1

INTRODUCTION

1.1 General introduction

Patterns of temporal variation of a community are important in describing the ecological diversity of a population over a period of time. Phytoplankton and benthic microalgae present as main algal biomass in the pelagic ecosystems (Paerl *et al.,* 2006). Therefore, they play an important role in the functioning of the marine trophic web. Increment or decrement of phytoplankton and benthic microalgae are affected by biotic and abiotic factors such as light, temperature, natural and anthropogenic nutrient sources, salinity and dissolved oxygen (Nowrouzi and Valavi, 2011). All of the above characteristics undertake temporal variations, which can occur on a short term (1 day to 1 week), mid-term (seasonal), or long term (inter-annual) basis. According to Chen and Liu (2010), phytoplankton temporal records are able to provide information on specific relation of phytoplankton with environmental variables such as biological, chemical and physical parameters. Hence, this study focuses on the taxonomical and ecological of phytoplankton and benthic microalgae over the period of 18 months.

1.2 Introduction to microalgae

Giordano and Raven (2014) stated that an alga can generally described as an organisms carrying out oxygen-producing (oxygenic) photosynthesis. The algae that range from the microscopic (microalgae) to large seaweeds (macroalgae). Microalgae, also known as microscopic algae are generally found both in marine and freshwater environments where they can live freely in the water column (phytoplankton) or attached to the sediment (benthic microalgae). The microalgae include diatoms, dinoflagellates, cyanobacteria, cryptomonads, golden algae, yellow- green algae, green algae, red algae, brown algae, euglenoids, haptophyte, cryptophyceae, glaucophyte, eustigmatophyte and raphidophyte (Wetzel, 2001) which exist individually, or in chains or groups.

The term phytoplankton represents the photoautotrophic component of single-celled microorganisms passively drifting within the water column. The habitat of phytoplankton extend from the upper, sunlight region of the pelagic ocean (euphotic zone) towards benthic environment as hydrodynamic processes carry phytoplankton present in seawater to the intertidal sediment (Shane & Joe, 2009). The total global primary production in the ocean are dominated by unicellular phytoplankton (Falkowski *et al*., 2004) and within the phytoplankton, the diatom generated approximately 40% to 45 % of net oceanic productivity (Falciatore & Bowler, 2002; Sarthou *et al*., 2005).

Benthic microalgae also known as microphytobenthos, refers to microscopic, unicellular photoautrotrophs which inhabit the upper centimeters of shelf sediments (Pinckney, 2018). According to MacIntyre *et al.* (1996), benthic microalgae are found growing in habitats ranging from wave swept beaches to detritus-laden backwater lagoons, and includes habitats such as salt marshes, submerged aquatic vegetation beds, intertidal sand and mud flats, and sub-tidal, illuminated sediments. Intertidal habitats in intertidal and shallow sub-tidal marine ecosystems frequently support extensive populations of benthic microalgae. They normally appear in greenish or brownish colour. Major component of benthic microalgae are the benthic diatom (de Bower *et al.*, 2003).

Phytoplankton are classified according to their individual sizes. Nanoplankton has been used to described plankton of size between 2 and 20 µm. Organisms with size range between 20 and 200 µm were refer as microplankton, while for organisms that has size range more than 200 µm the term macroplankton is applied. For smaller size plankton which range between 0.2 – 2.0 µm, they are classified as picoplankton (Finkel *et al.*, 2010). There are also chain forming diatom which can be several milimeters in length.

1.2.1 Diatom

Diatoms have been studied since the late 18th century. They belong to the class Bacillariophyceae from the phylum Bacillariophyta (Tomas, 1996). Diatoms are ubiquitous and they specifically require silica to grow and develop their cell wall (Dawes, 1998). The crucial factor that differentiate diatom from other types of algae is its frustule which form two overlapping valves made up of silica (SiO_2). Upper part of the cell is known as the epitheca while the lower part known as hypotheca (Tomas, 1996). This diatom cell can be seen in two different view, the valve view and girdle view.

There are two main categories of frustule shapes that can be distinguished, discoid or cylindrical cells with radial symmetry in valve view (centric diatoms) and elongated cells with a more or less bilateral symmetry (pennate diatom) (Brinkmann *et al.,* 2011). Most of the pennate diatoms have slits in their frustule that extends between both cell ends, the raphe system. Such diatoms are known as raphid diatoms and are capable of accomplishing rapid gliding motility (Round *et al.,* 1990; Graham *et al.,* 2009). On the other hand, pennate diatoms lacking with raphes are known as araphid diatoms (Round *et al.,* 1990; Graham *et al.,* 2009).

According to Agusti (2015), in cold waters, diatom dominate primary producers and although they are not dominant in tropical waters, they still play a major role in the biological pump and biogeochemical processes and normally occur as single cell (solitary) or in chain form (colonial). Size of single cell diatom can range from approximately 2µm to 2 mm, but for chains diatom the size can be several milimeters in length (Tomas, 1996).

Diatom dominates the phytoplankton and benthic microalgae communities in the coastal marine environment. The growth and behaviour of the diatom community structure in the pelagic and benthic environment is controlled by various environmental conditions. According to Tyler *et al*. (2003), benthic microalgae play a significant role in regulating carbon and nutrient turnover in shallow-water area, while phytoplankton often play a minor role aside from highly enriched systems (Valiela *et al.,* 1997). In benthic environment, diatom grow on different substrates such as sand (epipsammic), silt or mud (epipelic) and on stones or other hard surfaces (epilithic). The diatom can also be found living on other plants (epiphytic) and on animals (epizoon) (Round *et al.,* 1990). Benthic diatom can migrate to plankton habitat due to current and winds, this are known as tychoplanktonic species (Round *et al.,* 1990).

1.2.2 Dinoflagellate

Dinoflagellates are a diverse group of phytoplankton. According to Hoppenrath *et al.* (2014), majority of dinoflagellates are planktonic and only a small percentage is benthic. Dinoflagellates are motile eukaryotic organisms. There are approximately 2000 living dinoflagellates recorded worldwide (Taylor *et al.*, 2008) that have successfully adapted to a variety of pelagic and benthic habitats. Presents of normal population of dinoflagellates is permissible, however it becomes nuisance when the dinoflagellates outgrow the normal population.

Dinoflagellates have the ability to induce most of the endemic seafood poisoning. In toxic red tides, the dinoflagellates produce a chemical that act as a neurotoxin in other animals. For example, when the dinoflagellates were ingested by shellfish, the chemicals accumulate in the shellfish tissue and cause serious neurological affects in birds, animals, or humans which ingest the shellfish. In Malaysia, the outbreak of seafood poisoning was recorded since 1976 and they are mainly caused by *Pyrodinium bahamense* (Lim *et al.*, 2012, Adam *et al.*, 2011).

Dinoflagellates are divided into two groups based on their external morphology which are the armoured and naked dinoflagellates (Dodge, 1972). Theca of the armoured dinoflagellates are made up of polygonal cellulosic plates that cover the entire cell (Sumbali & Mehrotra, 2009). The number and arrangement of plates are used to differentiate the dinoflagellates between genera and species (Sumbali & Mehrotra, 2009). The other group is the althecate (naked) dinoflagellates where they lacked plates (Dodge, 1972). Other significant characteristic that can be used for identification is the basic cell shapes of dinoflagellates (Sarjeant, 1974). Two dissimilar flagella emerging anteriorly are known as desmokont while two dissimilar flagella emerging ventrally are known as dinokont. Desmokont flagella lack furrow while dinokont cell is split into two different parts, an epicone and a hypocone. Epicone and hypocone are separated by a groove called cingulum (girdle) (Tomas, 1996). A dinokont cell can either be thecate or athecate (Lee, 1999). As a unicell, the dinoflagellates size can range from 2 µm to 2000 µm.

Benthic dinoflagellates species are normally found in the interstitial spaces of marine sediments (sand dwelling) and in the intertidal shallow zone. They also live epiphytically on macroalgae and corals in the intertidal and subtidal zones (Hoppenrath *et al.*, 2014). There are some benthic- epiphytic dinoflagellates species that produce toxins (Antonio *et al.*, 2015). Consumption of this marine products that is contaminated with these compounds have bad results on public health (Antonio *et al.*, 2015). Benthic photosynthetic dinoflagellates species adapt in low light condition due to the interstitial spaces that receive only small amount of light compare to planktonic dinoflagellates.

1.3 Problem Statement

Teluk Bahang area faces some threat from both land and sea based pollution. Presents of aquaculture farm near the Penang National Park has also become one of cause of pollution in Teluk Bahang area. Phytoplankton and benthic microalgae have rapid response to physical parameters and nutrient enrichment. The co-existence of phytoplankton and benthic microalgae assemblies under common environmental conditions makes it possible to classify functional groups of organisms with similar morphological, physiological and biochemical characteristics or other defining characteristics (Alves- De Souza *et al.*, 2008; Roselli and Basset, 2015). Major taxonomic classes such diatoms, dinoflagellates and cyanobacteria are distinct functional groups, as these taxonomic groups tend to vary in their nutrient absorption and growth parameters, all of which are translated into different ecological strategies (Litchman *et al.,* 2007).

Study conduct by Stanca and Parsons (2017) recorded changes displayed by dinoflagellates and cyanobacteria along an environmental gradient consisting of changing light, temperature and wave energy. However, diatoms did not exhibit clear gradient relationships, possibly reflecting the high diversity within this phylum, including differences in cell shape and size which enable this group to adapt with many different but specific environmental regimes (Stanca and Parsons, 2017).

Long term effect of environmental conditions is likely to be reflected in the functional groups of benthic microalgae and phytoplankton. Therefore, it is crucial to monitor the diversity and ecology of phytoplankton and benthic microalgae in Teluk Bahang.

1.4 Objectives

There are two objectives in this study:

a) To characterize the phytoplankton and benthic microalgae community in the coastal areas of Teluk Bahang.

b) To identify the important physical and chemical factors that cause alteration in dominant phytoplankton and benthic microalgae over appropriate temporal scales.

1.5 Hypothesis

The hypothesis that are going to be tested in this study are:

a) Both planktonic and benthic microalgae will display different morphological characteristics.

b) Different environmental condition can affect the diversity and abundance of dominant phytoplankton and benthic microalgae community.

CHAPTER 2

LITERATURE REVIEW

2.1 Importance of taxonomy for ecological studies.

According to Warwick, 1988, in environmental impact assessment, a low taxonomic resolution could clearly indicate environmental pollution gradients. This is due tospecies that are more effected than higher taxa by both natural variability and seasonal cycles. Phytoplankton are widely disperse, therefore the assemblages are expected to be mainly shaped by local conditions (Beisner *et al.,* 2006). The conditions should thus support the group of species that offer similar adaptive features (Webb *et al.,* 2002).

Taxonomical and morphological characteristics of diatoms are normally used for bio-monitoring purposes at community, population and individual levels (Shristy *et al.,* 2017). In Teluk Bahang, there are many environmental variables to which growth rates of phytoplankton and benthic microalgae might respond. Choices of the taxonomic level is to optimise the efficiency rate in environmental studies, which might be related to the groups of organisms involved and to their distribution in the studied area (Terlizzi *et al*., 2003).

Biogeographical features and internal diversity of taxa have influence towards response of different taxonomic levels (Roy *et al.,* 1996). For example, if the family richness is a good alternative of species diversity in the North Sea, it might not be the same for the Mediterranean Sea (Bianchi and Morri, 2000). Silicified diatomaceous cell walls may respond quickly and characteristically against chemical contamination (organic and inorganic), which may be demonstrated as alterations in their morphology, for example modification in cell size, frustule outline, and raphe and striae patterns (Falasco *et al.*, 2009; Pandey *et al.*, 2014).

2.2 Influence of environmental parameters on phytoplankton and benthic microalgae

a) Nutrients

Pulau Pinang is one of the most develop state in Malaysia and centre of economic activities with very little remaining forests. Pulau Pinang National Park is the smallest national gazetted park in Malaysia in 2003 with only 1200 hectares (Kumar, 2004). The Penang National Park coastline face many treats such as environmental pollution, waste from aquaculture farm, and tourism development. These threats cause deterioration of marine and coastal habitats, resources and on the human health. Deviation from typical phytoplankton and benthic microalgae abundance and compositional patterns through temporal change can be used to detect the ecological changes happening in Teluk Bahang. This is due to role of phytoplankton and benthic microalgae as good indicator of the trophic status and environmental quality (Silva *et al.*, 2005).

Stolte *et al.* (1994) noted that due to small size of phytoplankton, phytoplankton become responsive towards variations in environmental conditions such as rapid nutrient uptake, and specific growth requirements. They play an important role in bio- monitoring of pollution and reflect the nutrient status of the particular environment or ecosystem (Davies *et al.*, 2009; Abowei *et al.*, 2012).

Nitrate is one of the limiting factors for phytoplankton growth and it is fundamental in structuring the phytoplankton community composition (Xu *et al.*, 2010). In Marudu bay, Sabah, Tan and Ransangan (2017) reported, *Chaetoceros* spp. and *Bacteriastrum* spp. grow rapidly when nitrate is available. According to Tanaka and Choo (2000), due to nutrients outwelling and tidal mixing in Matang mangrove estuary, high phytoplankton biomass during spring tide was observed.

Flint & Kamykowski (1984) and Ragueneau *et al.* (1994) stated that one of the important factors controlling the supply of nutrients to the water column is the nutrient regeneration in the sediments and phytoplankton abundance in shallow water. Increment in sediment nutrient levels results in higher benthic diatom biomass in muddy substrates, as the growth of benthic diatoms are supported by the nutrients (Cahoon & Safi, 2002). However, according to Mitbavkar & Anil (2002), presence of pennate diatom in Dias beach sandflats did not show any significant effects on nutrient level, but they found that the abundance of centric diatom was affected by nitrate and phosphate.

According to Sündback & Snoeijis (1991), sedimentation of biogenic particles is the primary source of regenerated material in coastal ecosystems. Breakdown of these particles produce produce organic and inorganic forms of nutrients that are available for direct uptake by planktonic and benthic autotrophs (Sündback *et al.*, 2000). The regenerated nutrient might be intercepted by benthic autotrophs, decoupling the supply of nutrient to the pelagic ecosystem (Krom, 1991). Besides nitrogen and phosphorus, silica has also been identified as a significant and probably as a limiting nutrient for the growth of microalgae (Ragueneau *et al.*, 1994).

b) Salinity

A small change in salinity can affect the composition of phytoplankton communities (Floder *et al.*, 2010). According to Kirst (1989), interspecific differences in salinity tolerance of phytoplankton play a major role in structuring phytoplankton communities whenever there is fluctuation in salinity. Similar study in Changjiang Estuary, China showed that, phytoplankton composition and dominant species changed with salinity (Gao & Song, 2005). *Melosira granulata* and most of the Chlorophyta species dominated water column with salinity less than 1 ppt (Gao & Song, 2005). While *Skeletonema costatum* dominated water column with salinity more than 25 ppt (Gao & Song, 2005). Jalal *et al.*, (2011) reported that the phytoplankton community in Pahang estuary was diverse during monsoon and dominant in non-monsoon season and fluctuation in salinity (0.02 – 27.01 ppt) have had an effect on most of the phytoplankton species present in the estuary.

Salinity gradient was one of the main components that contribute to the growth and diversity of phytoplankton communities in coastal waters and estuary (Khatoon *et al.*, 2010). The coastal species are normally more euryhaline than oceanic species, because salinity is less incline to radical fluctuations offshore than in coastal and estuarine waters. According to Nursuhayati *et al.* (2013), marine phytoplankton is adapted to high salinity and usually are not able to survive in freshwater environment. Nevertheless, there are also some marine and freshwater species can tolerate and survive in high salinity fluctuations (Nursuhayati *et al*, 2013).

Blasutto *et al.* (2005) observed that benthic diatom produces high primary production in higher salinity (32.1 ppt) conditions. Underwood *et al.* (1999) reported that the relative abundance of several diatom species shifted along the salinity gradient and from the study, it shows that the diatom genera shifted along the oligo and meso and polyhaline sites, with the abundant of *Navicula gregaria* and *Navicula phyllepta* at the low to mid salinity range while other species like *Pleurosigma angulatum* and *Plagiotropis vitrea* can be found at high salinity gradient (> 30 ppt).

c) Temperature

Fluctuations of intertidal sediment temperature occurs on long (seasonal) and short (daily and hourly) time scales, depending on factors such as meteorological conditions, time of day and tidal inundation (Vieira *et al.,* 2013). Short term temperature shift, like those experienced by intertidal benthic microalgae communities during tidal cycle, have been shown to have significant effect on the photosynthesis of cultured benthic diatoms (*Amphora cf coffeaeformis* and *Cocconeis sublittoral*) and suspension of intertidal benthic microalgae (Morris and Kromkamp, 2003; Salleh and McMinn, 2011, Blanchard *et al.*, 1997). Blanchard *et al*. (1997) stated that during short term acclimation to elevated temperatures some microalgae were able to decrease their photosynthetic rates, while long term exposure led to variation in the species composition of the microalgal community inhabiting the sediment surface (Defew *et al*., 2004).

Effect of temperature varies for communities of different composition due to different algal species and groups differ in their temperature optima (Thomas *et al.,* 2012). Nevertheless, variation in temperature can have positive or negative effects on phytoplankton biomass such as a long-term increment in temperature (1 °C) can increase the primary production in lakes whereas the same temperature increment can decrease the marine phytoplankton biomass (Boyce *et al.*, 2010; Gerten and Adrian, 2002).

d) pH

According to Chen and Edward (1994), changes in pH can affect algal growth in a few ways by changing the distribution of carbon dioxide and carbon availability. Variation in pH levels in marine environments appear to correlate with changes in temperature, dissolved oxygen and phytoplankton production (Chen and Edward, 1994). Event of global warming has caused the surface waters to be more vulnerable to acidification with the increment of carbon dioxide concentrations in the atmosphere (Olivia and Joe, 2017). According to Chu (2015), anthropogenic carbon dioxide from the burning of fossil fuels has cause increment in the uptake of greenhouse gas which lead to the pH to drop from 8.2 to 8.1 in ocean. There are many harmful effects to the marine life in oceans and the issue will continue to take place unless the carbon dioxide decreases. This might also result in changes of species abundance and diversity of phytoplankton (Fabry *et al.*, 2008). *Chaetoceros calcitrans* and *Skeletonema costatum* were isolated from samples collected from West Coast of India. Both species prefer neutral to alkaline pH where they showed maximum growth at pH 7.5 (Sushanth and Rajashekhar, 2014).

According to Riley & Chester (1971), it is generally assumed that pH in marine environments change little around a typical surface value of 8.2. Diatoms have been reported to grow optimally near pH 8.1 and exhibit a notable decrease in their growth rate at pH 8.5 (Hinga, 1992). Most phytoplankton species possess CO_2 concentrating mechanisms which increase free CO_2 in the vicinity of RUBISCO (Giordano *et al*, 2005). However, a pH drop of ~0.4 units, as predicted, represents a ~150% increment in the H^+ concentration which might affect the intracellular pH, membrane potential, energy portioning and enzyme activity (Beardall & Raven, 2004; Riebesell, 2004). Therefore, ocean acidification might lower phytoplankton growth rates through pH effects (Berge *et al.*, 2010). According to Johnson *et al.* (2011), increase in CO_2 can stimulate growth of benthic diatom species, particularly large, chain forming genera, promoting the primary productivity in shallow water coastal habitats.

e) Sediment

According to Watermann *et al.* (1999), sediment grains size plays an important role in the distribution and abundance of benthic microalgae. Type of sediment have strong impact on the chemical and physical conditions within the sediment, which is indicate type of benthic microalgae found (Paterson and Hagerthey, 2001).

Mitbavkar & Anil (2006) reported that sediment grain size differs with the months. The changes indicate that the grain size could serve as a predictor of *Grammatophora* (500 µm grain size) at 0 -5 cm, and *Achnanthes*, *Biddulphia*, *Melosira*, *Streptotheca* (250 µm grain size fraction), *Cyclotella* (500 and 125 µm grain size fraction), *Pinnularia* (63 µm grain size fraction) and *Coscinodiscus* (500, 125 and 63 µm grain size fraction) at the 10 – 15 cm depth.

During low tide, the benthic microalgae community will be exposed to direct sunlight for longer hours compared to during the high tide. Benthic microalgae photosynthesis in muddy sediments is limited to thin photic zone (<2mm) which favours benthic microalgae such as epipelic diatom with high migration and cell division rates over other populations (Pinckney, 1994). Cahoon *et al.* (1999) reported increment in chlorophyll *a* biomass content was found in sediment that contain fewer fine particles (<125mm). There was insignificant relationship between benthic microalgae biomass and fraction of fine grain size. This might occur due to increase in anthropogenic loadings of fine grain sizes uptake (Cahoon *et al.,* 1999)

2.3 Distribution of microalgae

a) Benthic Microalgae

There are studies conducted in detail on the microalgae productivity and microalgae taxonomy (Boonyapiwat, 1999) of Peninsula Malaysia. On the other hand, little is known for benthic diatom. In Malaysia, benthic studies on freshwater diatom has been conducted by Wan Maznah and Mansor (2002) in the Penang river basin, apart from a study done in Sarawak Rivers (Hilalludin *et al.,* 2011). Limited studies have been conducted on diatom inhabiting coastal environments. Studies conducted by Cheng (2013) in Penang, reported the presence of benthic microalgae in Tanjung Bungah and Pantai Jerejak. While in another study conducted by McMinn *et al.* (2005), the authors focused on assemblages of benthic microalgae in Muka Head and area nearby Songsong Islands, Penang. The authors reported the occurrence of diatom from the genera *Cocconeis, Fragilaria, Paralia* and *Pleurosigma.* Alia (2017) reported the existence of 51 species of benthic microalgae in the coastal area of Tanjung Bungah and 56 species of benthic microalgae in Tanjung Rhu estuary. Episammic diatom were reported to dominate Tanjung Bungah and Tanjung Rhu. Tanjung Bungah coastal area was dominated by *Navicula* and *Thalassiosira*, while Tanjung Rhu estuary was dominated by *Amphora* and *Cocconeis*.

b) Phytoplankton

Various studies on the distribution and ecology of phytoplankton have been conducted in the tropical area of Peninsular Malaysia. According to Salleh *et al*. (2005), a total of 29 genus of phytoplankton were found in Langkawi where the dominant genera belongs to *Chaetoceros*. Similar dominant genera which is *Chaetoceros* were also reported in Straits of Malacca (Salleh *et al.,* 2008). Shamsudin *et al.,* (1987) suggested that the most abundance phytoplankton found in coasts of Kelantan, Terengganu and Johore are diatoms with numerous species such as *Pleurosigma* and *Chaetoceros*. While in Paya Bungor, Pahang Chlorophyta where reported as the most diverse phytoplankton in the tropical lake and Cyanophyta was the most abundant phytoplankton group. Muhammad Adlan *et al.* (2012) reported there are several group of phytoplankton that can be found in coastal waters of Manjung, Malaysia such as Bacillariophyta, Cynaphyta, Chlorophyta and Dinophyta and the dominant species found was *Odontella sinensis*. However, futher studies of the phytoplankton based on temporal scales have not been implemented in these tropical areas specifically.

CHAPTER 3

METHODOLOGY

3.1 Study Area

Teluk Bahang is a fishing village located in northern of Pulau Pinang. It is a well-known location for hiking, trekking and tourist activities. Three study sites (Figure 3.0) have been selected for this study; Station A (5°27´47.80´´N, 100°12´10.56´´E), Station B (5°27´46.05´´N, 100°12´11.48´´E) and Station C (5°27´ 44.36´´N, 100°12´ 27.07´´E). Station A and Station B are located at the intertidal area of Pasir Pandak, Teluk Bahang (Figure 3.1a, b, c). Pasir Pandak is located at the very edge of the northern boundary of Penang National Park. Penang National Park is the only national park in Peninsular Malaysia with the coastal habitat that support diverse range of habitats from sandy and rocky shores to mangroves (Sara *et al.*, 2015). The intertidal area of Pasir Pandak is sheltered and covered with sandy beach and seasonal muddy seabed. At Station B, there is a stream (Sungai Tukun) that flows into the bay. This sometimes cause fluctuation in salinity value at Station B. Station A and B faced anthropogenic disturbance coming from the aquaculture farm operating about 500 meters away from the intertidal area. Station C is a sub-tidal area located near the Teluk Bahang jetty (Figure 3.1 c). The jetty area was also exposed to oil and waste from the tourist boats and the fishing trawlers. Samplings were conducted monthly during low tide for eighteen months from January 2015 until June 2016.

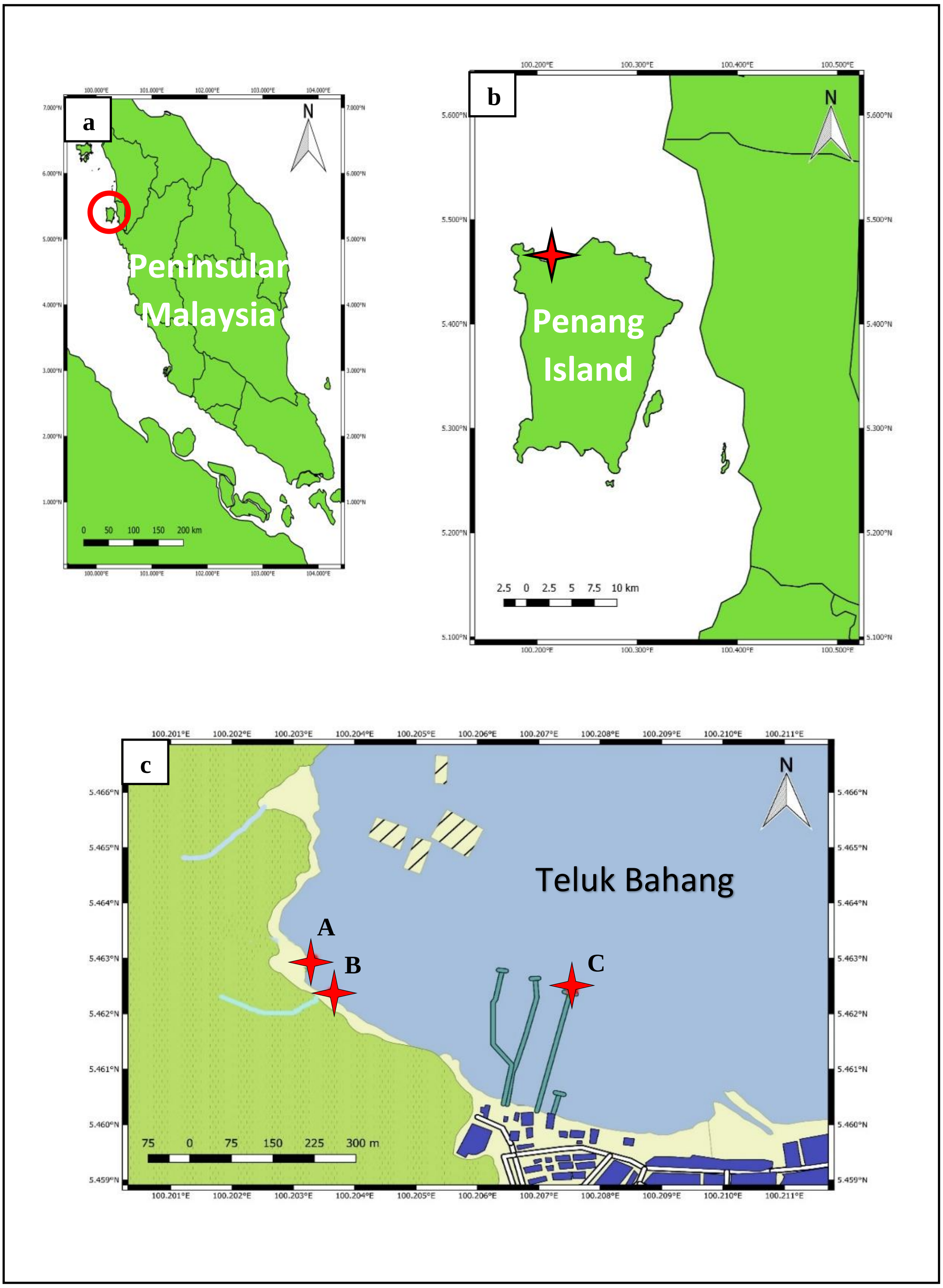

Figure 3.0: Map of Peninsular Malaysia showing Penang Island and Teluk Bahang. a) Map of Peninsular Malaysia. The round symbol (○) indicates Penang Island. b) Map of Penang Island. c) Map of Teluk Bahang. The red star symbols (✦) indicates the sampling sites.

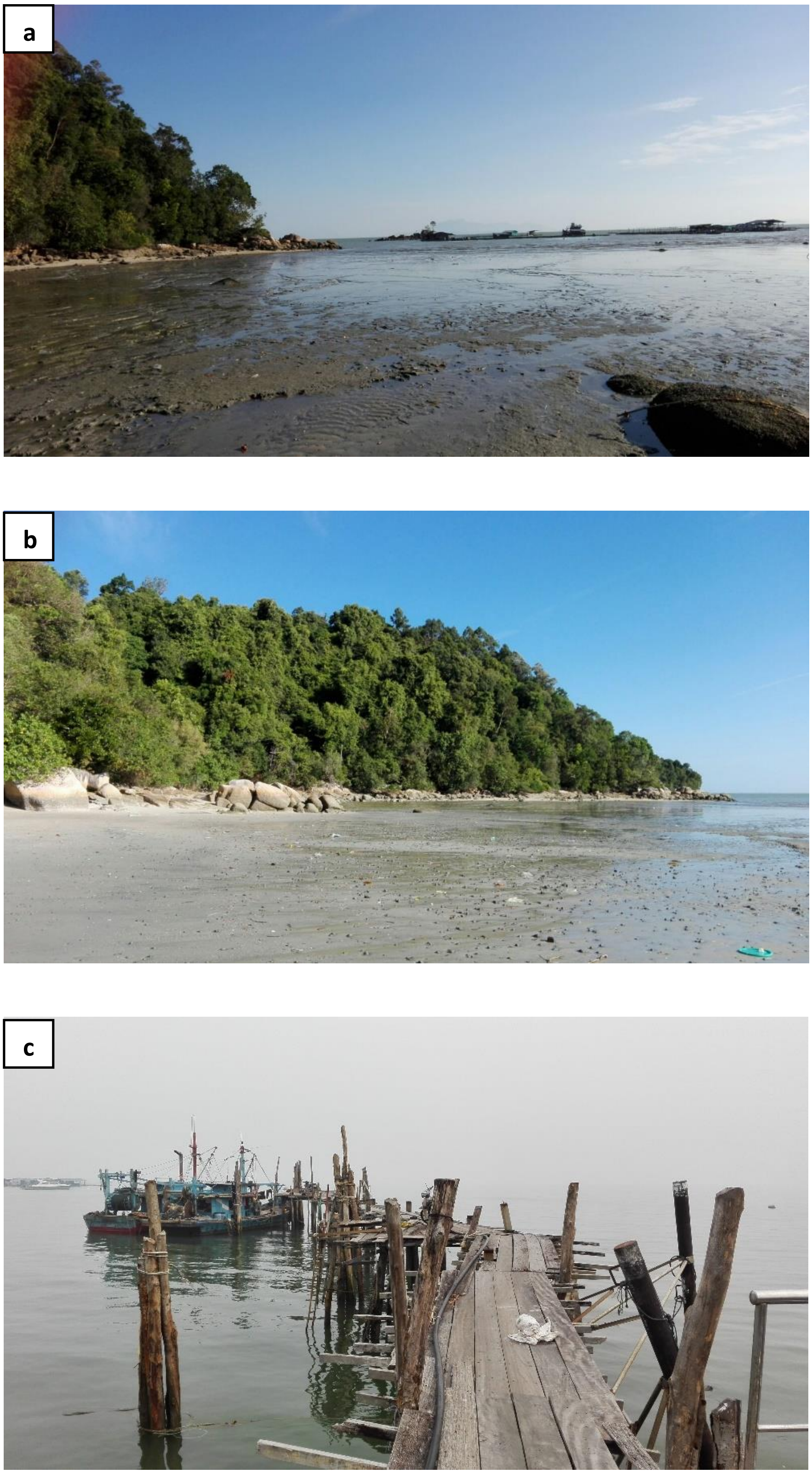

Figure 3.1: Three sampling stations. a) Station A is a sheltered area composed mainly of sandy and gravel sediments. b) Station B There is also presents of stream (Sungai Tukun) that flows into the bay. c) Station C is a jetty area disturbed by anthropogenic sources introduced to the underlying water body.

3.2 Sampling Strategy

Sample collections were carried out during low tide along the intertidal and jetty areas according to coordinates (Station A, B and C) in Teluk Bahang for 18 months. Benthic microalgae samples (n=3) were collected from surface layer of sediment, 5 mm of depth and the samples collected were stored in 50 ml centrifuge tube. The benthic microalgae samples were added with 20 ml filtered seawater before adding Lugols' solution to preserve the samples. Samples were gently shaken after adding Lugols' solution.

The phytoplankton samples were collected using 35 µm mesh-size plankton net at the jetty (Station C). Five liters of water samples were filtered through the plankton net to acquire the plankton samples (n=3) (Wood, 1962). The concentrated phytoplankton samples collected were stored in 50 ml centrifuge tube and were preserved by adding a few drops of Lugols' solution. Samples change accordingly after adding Lugols' solution.

3.3 Rainfall data

According to the Malaysian Meteorological Department (MMD) annual report, rainfall above 200mm represent the wet season and 0-200 mm represents the dry season (MMD, 2009). This study used rainfall data from MMD 2015-2016 and adopted seasonal classifications used by MMD for grouping the data into dry and wet seasons (Figure 3.2). The mean rainfall for 18 months at Teluk Bahang was 2956.5 mm during the study period.

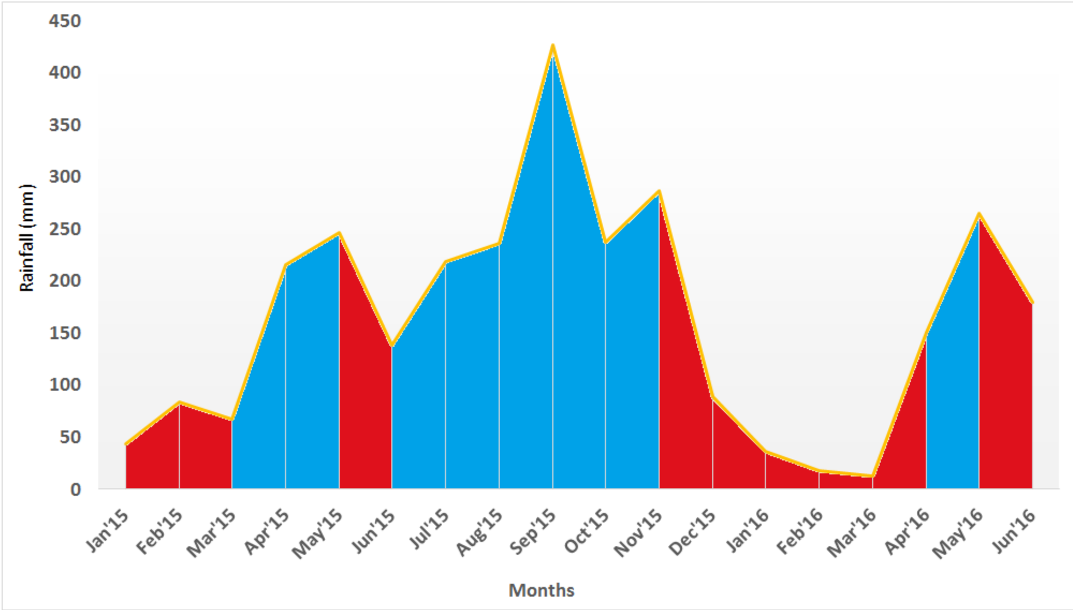

Figure 3.2: Mean rainfall (mm) in Teluk Bahang. Red shaded area indicates dry season, blue shaded area indicates wet season.

3.4 Physical parameters

Physical parameters such as temperature (°C), salinity, dissolved oxygen (mg/L) and pH were recorded at *in-situ*. Measurements (n=3) were done using YSI 5100 dissolved oxygen meter for dissolved oxygen parameter, Metler Toledo pH meter, U.S.A. for pH parameter and Atago hand held refractometer, U.S.A. for salinity parameter. The sampling method was adapted from Riberio *et al.* (2013).

3.5 Laboratory analyses

3.5.1 Phytoplankton and benthic microalgae preparation, identification and enumeration

Benthic diatom samples were collected at each sampling site by scraping the top 5 mm of the intertidal sediments (n=3). All samples collected was stored in 50 mL centrifuge tube. A total of 20 mL filtered seawater was added to the mixture for dilution. The samples were preserved using 0.5% Lugol's solution by adding a few drops into the mixture and shaking them lightly.

Approximately 5 ml of phytoplankton and benthic samples were transferred to another 15 mL centrifuge tube, where organic matter in the mixture was removed from the sediments by digestion in 10% hydrogen peroxide and was left for 72 hours. The mixture was then rinsed with distilled water to remove excess hydrogen peroxide by removing the suspended solution. This rinsing process was repeated several times to obtain a clean sample for microscope viewing. The cleaned samples were stored in the refrigerator (4 °C) until analysis were conducted.

Further cleaning of phytoplankton and benthic microalgae were done for SEM analysis. The samples were filtered using filtration set with isopore membrane filters (0.4 µm). The cleaned samples were viewed under the Carl Zeiss SMT (Germany), Leo Supra 50 VP Field Emission Scanning Electron Microscope. Clear SEM images of phytoplankton and benthic microalgae were used for identification. Identification of diatoms and dinoflagellates to species level was based on general morphological description of phytoplankton as in literature (Omura and Takuo, 2012 and Hallegraeff *et al.* 2010).

For cell enumeration, triplicate sub samples (n=3) were taken for cell counts. One millimeter of concentrated phytoplankton and benthic microalgae was placed in a Sedgewick-Rafter counting chamber, observed and enumerated under light microscope. Cell densities, in the unit of cells L^{-1}, were measured from triplicate counts and presented as mean (± standard deviation).

3.5.2 Chlorophyll *a* biomass

a) Pore and surface water chlorophyll a

Pore water samples for Station A and B were taken from underlying interstitial water in sediments using a 250 mL polyethylene bottle (n=3). Surface seawater were taken using van Dorn water sampler at Station C near the jetty. All the samples were collected during low tide, monthly for 18 months. 100 ml of water samples (pore water and surface seawater) were filtered through a Whatmann GF/C glass microfiber filter paper, U.K. (47 in diameter, 1.2 µm in pore size) coupled with a milipore pump to remove the suspended solids. The filter paper was taken out from filtration unit using forceps to avoid contamination and placed in centrifuge tube (15 ml). 10 ml of methanol (90%) was added into the centrifuge tube and was crushed with a glass rod before covering with aluminum foil and placed in fridge (4°C) for overnight (Strickland and Parson, 1986). Then, the tube was centrifuge at 2000- rpm for 10 minutes. The absorbance of supernatant was recorded at 750, 665, 645 and 630 nm by using the SHIMADZU UVmini-1240, UV-VIS Spectophotometer (Japan) with 1 cm quartz cuvette. The 90% methanol was used as blank. The Strickland and Parson, (1986) formula as quoted in Hing *et al.*, (2012) was used in calculating chlorophyll *α* concentration as follows:

$$\text{Chl } \alpha \text{ (mg/m}^3) = [(11.6 \cdot D_{665\text{-}750} - 1.31 \cdot D_{645\text{-}750} - 0.41 \cdot D_{630\text{-}750}) \cdot V_e] / (L \cdot V_f)$$

Where,

L = Cuvette light-path (cm)

V_e = Extration volume (ml)

V_f = Filtered volume (l)

b) Benthic chlorophyll *a*

Top 5 mm of the sediment surface (n=3) were collected using 15 mL centrifuge tube for benthic chlorophyll α analysis. The 90% methanol (10 ml) was added into the sediment. Then, the centrifuge tube was wrap with aluminum foil and kept in dark and freeze at 0-4 °C for 24 hours. The tube was centrifuged for 10 minutes at 2000- rpm. Absorbance of supernatant were taken at 750, 665, 645 and 630 nm by using the SHIMADZU UVmini-1240, UV-VIS Spectophotometer (Japan) using 1 cm quartz cuvette. Blank used is the 90% methanol. The formula for calculating the benthic chlorophyll α was modified from Lorenzen (1967), as follows:

$$\text{Chl } \alpha \ (\text{mg/m}^2) = [(11.6 \bullet D_{665\text{-}750} - 1.31 \bullet D_{645\text{-}750} - 0.41 \bullet D_{630\text{-}750}) \bullet V_e] / (L \bullet A_c)$$

Where,

L = Cuvette light-path (cm)

V_e = Extration volume (ml)

A_c = Area of core (l8.85 m^2)

3.5.3 Nutrient analysis

To determine nutrient conditions during field samplings at Teluk Bahang, nutrient analyes of silica, nitrite, nitrate, ortho-phosphate and ammonia were carried out using standard titration method modified from Strickland and Parsons (1972) and Hing *et al.* (2012). A spectrophotometer (UVmini-1240, Shimadzu, Japan) was used to measure the absorbance of the solution using a 1cm quartz cuvette and distilled