PHOTOPHYSIOLOGY AND PHOTOPROTECTION OF MARINE MICROALGAE COMMUNITIES IN NORTHERN STRAITS OF MALACCA AND ANTARCTIC PENINSULA

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF SYMBOLS AND ABBREVIATION

a	photosynthetic efficiency
ANOVA	Analysis of Variance
ATP	adenosine triphosphate
CEMACS	Centre for Marine and Coastal Studies
Chl a	chlorophyll *a*
cell/L	cell per litre
DO	dissolved oxygen
E_k	photoacclimation index
F_v/F_m	maximum quantum yield
F_v'/F_m'	effective quantum yield
mg/L	milligram per litre
NADPH	nicotinamide adenine dinucleotide phosphate
NH_3	ammonia
NOx	nitrate/nitrite
NPQ	non-photochemical quenching
PAM	Pulse Amplitude Modulation
P-E	photosynthesis – irradiance
PO_4^{2-}	ortho-phosphate
ppt	parts per thousand
$rETR_{max}$	maximal electron transport rate
rETR	relative electron transport rate
RLCs	rapid light curves
Si	silica
SST	sea surface temperature
USM	Universiti Sains Malaysia
° C	degree Celsius
µg/L	microgram per litre
µmol m^{-2} s^{-1}	micro mol per meter squared per second

LIST OF APPENDICES

FOTO-FISIOLOGI DAN FOTO-PERLINDUNGAN TERHADAP KOMUNITI MIKROALGA MARIN DI UTARA SELAT MELAKA DAN SEMENANJUNG ANTARTIKA

ABSTRAK

Mikroalga marin memainkan peranan penting dalam daya pengeluaran sejagat di ekosistem marin pantai. Pengenalan fluorometer aktif seperti "Pulse Amplitude Modulation" (PAM) dapat mengukur radas fotosintesis di lapangan. Ini memberikan pemahaman tentang tindakbalas mikroalga terhadap kondisi persekitaran dan nutrien. Tesis ini mengkaji struktur komuniti dan keadaan foto-fisiologi ke atas komuniti mikroalga tropikal dan polar dan tindakbalasnya terhadap kepelbagaian kecerunan iradians. Antara hipotesis kajian adalah komposisi mikroalga di tropikal dan polar akan dipengaruhi oleh pembolehubah persekitaran. Persampelan telah dijalankan di lima stesen di utara Selat Melaka pada bulan November dan Disember 2015 (fasa lembap) dan Februari 2016 (fasa kering) dari CEMACS USM, Pulau Pinang hingga Pulau Songsong, Kedah. "Malaysian Antarctic Scientific Expedition" telah dijalankan selama sebulan pada 18 Januari - 8 Februari 2016 yang melibatkan lapan stesen persampelan dari Ushuaia, Argentina sehingga Pulau Darboux, Semenanjung Antartika. Ukuran *in situ* seperti parameter fizikal (kemasinan, cahaya dan suhu), biomas klorofil *a* dan nutrien (NOx, NH_3, PO_4^{3-}, and SiO_2) telah direkodkan. Sebanyak tiga puluh lapan spesis mikroalga tropika dan dua puluh dua spesis mikroalga polar telah dikenalpasti dan didominasi oleh diatom. *Lauderia annulata* dan *Coscinodiscus radiatus* mendominasi kawasan tropika dan *Odontella weissflogii, Coscinodiscus gracilis, Fragilariopsis curta* and *Navicula* sp. mendominasi di kawasan polar. Analisis koresponden kanonikal (CCA) menunjukkan parameter persekitaran mempunyai kesan yang lemah terhadap mikroalga tropikal dan polar di semua stesen.

Manakala regresi linear menunjukkan *Coscinodiscus radiatus* mempunyai kesan lemah terhadap cahaya (r adalah 0.203). Manakala regresi linear larutan NOx memberi kesan lemah kepada *Asterionella frauenfeldii* (r adalah 0.090). Mikroalga polar, diatom memusat mempunyai kesan lemah terhadap suhu (r adalah 0.024). Manakala, cahaya memberi kesan lemah terhadap diatom pennate (r adalah 0.037). Bagi menguji kesan foto-fisiologi mikroalga di persekitaran, *in situ* foto-fisiologi diukur menggunakan fluorometer "Pocket Pulse Amplitude Modulated" (PAM). Nilai hasil kuantum maksimum (F_v/F_m) yang tertinggi telah direkodkan di stesen Pulau Songsong pada November 2015 dengan 0.473±0.023 dan di stesen Pulau Darboux dengan nilai 0.291±0.00. Mikroalga tropika telah didedahkan dengan berlainan pancaran iradians dan mampu bertahan di kesemua pancaran iradians. Mikroalga polar mampu beradaptasi di pancaran iradians 10 µmol m^{-2} s^{-1} dan akan tetapi tidak bertahan di pancaran iradians 600 dan 1000 µmol m^{-2} s^{-1}. Peningkatan fotokimia tak pelindapan (NPQ) menunjukkan tenaga cahaya hilang sebagai haba dan teraktifnya fotoperlindungan. Kajian ini mencadangkan bahawa mikroalga tropikal dan polar tidak terlalu dipengaruhi dengan parameter persekitaran. Prestasi fotosintesis di kedua-dua kawasan berkurangan apabila iradians meningkat dan terdedah pada masa yang panjang kepada mikroalga tropikal dan polar menyebabkan komponen fotosintesis terjejas.

PHOTOPHYSIOLOGY AND PHOTOPROTECTION OF MARINE MICROALGAE COMMUNITIES IN NORTHERN STRAITS OF MALACCA AND ANTARCTIC PENINSULA

ABSTRACT

Marine microalgae play a significant role in global primary productivity in coastal marine ecosystem. The introduction of active fluorometer such as the Pulse Amplitude Modulation (PAM) fluorometer in the 1980s allows measurements photosynthetic parameters in the field. This provides valuable information of microalgae on their responses to different environmental and nutrient conditions. This thesis investigates the community structure and photo-physiology conditions of tropical and polar microalgae and their responses to varying irradiance regimes. It is hypothesized that microalgae composition in tropical and polar will be affected by various environmental variables. Sample was collected from five stations in the Northern Straits of Malacca was conducted in November and December 2015 (wet phase) and February 2016 (dry phase) which started from CEMACS USM, Pulau Pinang to Pulau Songsong, Kedah. Malaysian Antarctic Scientific Expedition was conducted for 1 month from 18 January to 8 February 2016 in Ushuaia, Argentina to Darboux Island, Antarctic Peninsula with eight sampling stations. *In-situ* measurements such as environmental parameters (salinity & temperature), chlorophyll *a* biomass, and nutrients (NOx, NH_3, PO_4^{3-}, and SiO_2) were taken during sampling. Thirty-eight tropical species and twenty-two polar marine microalgae species were identified, and these were dominated by diatoms. *Lauderia annulata* and *Coscinodiscus radiatus* were dominant in tropical region and *Odontella weissflogii, Coscinodiscus gracilis, Fragilariopsis curta* and *Navicula* sp. were dominant in the polar region. Canonical Correspondence Analysis (CCA) showed low interaction

between environmental parameter and tropical and polar microalgae however linear regression proved *Coscinodiscus radiatus* was weakly affected by light (r was 0.203). Another linear regression showed NOx weakly affected the distribution of *Asterionella frauenfeldii* (r was 0.090). Polar microalgae, centric diatom was weakly affected by temperature (r was 0.024). Light weakly affecting the distribution of pennate diatom (r was 0.037). To investigate the photo-physiology of microalgae in environment, i*n-situ* measurements were measured using Pocket Pulse Amplitude Modulated (PAM) fluorometer. In tropical region maximum quantum yield (F_v/F_m) was the highest in Pulau Songsong in wet phase with 0.473±0.023 and in polar Darboux Island station with 0.291±0.00. Tropical microalgae with exposed to different irradiance treatment were able to thrive in all irradiance. While polar microalgae well adapted to the 10 µmol m^{-2} s^{-1} irradiance treatment but unable to thrive in 600 and 1000 µmol m^{-2} s^{-1} in 23 hours exposure. Increase of non-photochemical quenching (NPQ) showed the light energy dissipated as heat and activated photoprotection. This study suggested that both tropical and polar microalgae was partially influenced by environmental parameter. Photosynthetic performance in both assemblages was reduced in elevated irradiance and longer exposure time towards tropical and polar due to damaged photosynthetic components.

CHAPTER 1

INTRODUCTION

1.1 General introduction

Global primary production for plant is estimated worth of 1.4 x 10^{14} kg annually. 40% of the annual global primary production are made up from variety of marine microalgae species (Golley, 1972). Microalgae are responsible for global significance for climate regulation and biogeochemical cycling (Winder & Sommer, 2012). Microalgae are extremely important for primary production, key to the health, productivity of marine ecosystem, and contributed to higher trophic levels food source availability (Petrou *et al*., 2016).

Algae are fundamentally oxygen-discharging photosynthetic life form with simple body plan – no roots, stems or leaves and are usually aquatic. Algal diversity may be estimated by their biochemical composition, ecological roles, endosymbiotic genomes, morphology, regenerative technique, and photosynthetic parameters (Armbrust, 2009; Lizotte & Sullivan, 1992). Microalgae were discovered growing naturally and their diversity and abundance often vary, relying on several factors such as nutrient availability, light intensity, temperature, and salinity. Marine microalgae can thrive in a wide range of habitats within sediment of intertidal areas such as estuaries, sand flats, muddy shores, brackish, marine and soil (Metting, 1996; Mohd Nasarudin & Othman, 2012; Suthers & Rissik, 2009). Composition and distribution of microalgae in tropical and polar region are essential as the primary food producer affecting the population of the food chain.

Pulse amplitude modulated (PAM) fluorometer is a tool used to closely monitor photo-physiological state of photosystem. It is widely used in terrestrial plants and

microalgae (Chazalon *et al.,* 2014; Ritchie & Bunthawin, 2010). It gained popularity due to its rapid measurement, non-destructive technique, and portable for measurement of photochemical efficiency and photosynthetic rate (Baker, 2008; Consalvey *et al.,* 2005).

The Malacca Straits is located between the east coast Island of Sumatera, Indonesia and Peninsular Malaysia west coast and connected to Straits of Singapore at south-east end (Thia-eng *et al*., 2000). Chlorophyll *a* concentration in the south and along the coastal of Malacca Straits is high (up to 2.0 mg/m^3) annually, and towards north the concentration is decreasing (Ku Kassim *et al.,* 2007). Meanwhile, Antarctic Peninsula is separated with South America by Drake Passage. The average primary production of the Western Antarctic Peninsula (WAP) is 182 g C m^{-2} y^{-1} annually and a productive region in the Southern Ocean (Trimborn *et al.,* 2015).

1.2 Overview of microalgae

Microalgae are microscopic algae found in freshwater and marine ecosystem (Venkatesan *et al.,* 2015). Microalgae can efficiently convert sunlight into chemical energy through photosynthesis. Microalgae can be found floating in the upper 200m of the ocean to receive sunlight for photosynthesis. Their sizes range from few micrometers to more than 100 micrometer (Lavens & Sorgeloos, 1996; Venkatesan *et al.*, 2015).. Chlorophyll is a major photosynthetic pigment and chlorophyll *a* is present in all microalgae. Other "accessory pigments" present in several types of microalgae such as chlorophylls *b, c,* and *d,* carotenoid, phycoerythrins, phycocynins, and xanthophylls which also needed before photosynthesis process happen. Marine microalgae comprise of eukaryotic photoautotrophs and prokaryotic cyanobacteria (blue-green algae) (Singh & Saxena, 2015). Cyanobacteria are organisms that have

some characteristic in both algae and bacteria. Different to other bacteria, cyanobacteria contain blue-green or green pigment that able to perform photosynthesis (WHO, 2003). Marine microalgae are mainly comprised of diatoms and dinoflagellates. Other groups are green algae, blue-green algae (cyanobacteria), and coccolithophoridae (Singh & Saxena, 2015).

Diatoms in Bacillariophyceae class can be found in both freshwater and marine environment, mainly in solitary and colonies. The term diatoms was derived from a Greek word *diatomos,* meaning "cut in half" which referred to the distinctive two-part cell walls made of silica (Armbrust, 2009). Diatoms are the most diverse group of phytoplankton with estimated of 200,000 different species (Armbrust, 2009). Most of the diatoms are microscopic with size ranging from 2 – 500 µm. The cell wall of the diatoms are composed of silica; hydrated glass ($SiO_2.n\ H_2O$) (Drum & Gordon, 2003). Centric diatom and pennate diatom are types of diatoms (Round *et al.*, 1990). Centric diatoms consist of round and polygonal valve and pennate diatoms have bipolar, elongated valve (Round *et al.*, 1990). Pennate diatoms mostly are benthic or epiphytic and can be found in environment which is rich in dissolve organic matter while centric diatoms are predominantly planktonic and found in open water with relatively low concentrations of dissolve organic matter (Werner, 1977). Diatoms tend to dominate phytoplankton communities in well-mixed coastal and upwelling regions, and also along the edge of the sea-ice, where ample light, inorganic nitrogen, phosphorus, silicon, and trace elements are sufficient to support their production. Larger diatom species can travel up and down through the water column by regulating its buoyancy. Some open-ocean species can shift between well-lit but nutrient-depleted surface waters where in they photosynthesize and nitrate-rich water at depth of approximately

100 m where they consume and store nutrients required to keep dividing (Armbrust, 2009).

Dinoflagellates or *Pyrrophyta* is a single celled organism with two flagella that can be manipulated for movement. One flagella is located at the groove called girdle, and the other at the posterior of the cell (Karleskint *et al.,* 2009; Scott & Marchant, 2005). Dinoflagellate are differentiated by "armoured" and "unarmoured" dinoflagellates by pattern of thecate or amphiesma that is made up from cellulose plate (Hamdan *et al.,* 2017). Dinoflagellates possess chlorophyll *a* and *c*, and accessory photosynthetic pigments β-carotene and peridinin within the primitive plastids of dinoflagellates. Most of photosynthetic dinoflagellates can be autotrophic, heterotrophic or mixotrophic (Hamdan *et al*., 2017; Karleskint *et al.*, 2009; Scott & Marchant, 2005). Heterotrophic type of dinoflagellates prey on other smaller microorganism due to lack of photosynthesis system. A type of dinoflagellates known as zooxanthellae are from a genus *Symbodinium* spp. Zooxanthellae form a symbiotic relationship between coral which the photosynthesis product from zooxanthellae is used by coral for metabolism, growth, reproduction and survival (Hamdan *et al.,* 2017).

1.3 Light reaction of photosynthesis

The light reactions process happens in thylakoid membrane and the inner membrane of the chloroplast. It is also characterized as the Z scheme (Figure 1.1). The light reactions process begins with oxygen is produced by the oxidation of oxygen and $NADP^+$ reduced to NADPH. Photophosphorylation occur when the series of redox reactions conjoin with phosphorylation of ADP to ATP (Karleskint *et al*., 2009). Light absorption and energy conversion are part of photosynthetic light reactions. As for light absorption, light energy is absorbed by chlorophyll as chlorophyll *a* is a major

pigment with absorbing red (650-700 nm) and blue (400-450 nm) wavelength. While for energy conversion, the electron is in the ground state of low stable electron orbital. Enough supply of energy from photons of light to the reaction centre boost the electron to the highest orbital as it called excitation. The loss of energy during de-excitation is transformed into heat.

Light reaction of photosynthesis is divided into two part. The reaction of reduction of $NADP^+$ to NADPH happen in photosystem I (PSI) and the second part is the reaction if oxidation of water to oxygen carried out in photosystem II (PSII). The photons energy absorbs reaches P680 (PSII) an electron to an excited state $P680^+$. Unstable molecule then passes electron from PSII to PSI through electron transport chain that consist of primary electron acceptor (phaeophytin), plastoquinone (Pq), complex of cytochrome and plastocyanin (Pc) (Consalvey *et al.*, 2005). The production of ATP happens along the electron transfer chain as it contributes to proton gradient through ATP synthetase. Plastocyanin (Pc) transport the electron to P700. Light energy collects from light harvesting complex pass to chlorophyll *a* molecule in reaction centre in which passes to the primary electron acceptor.

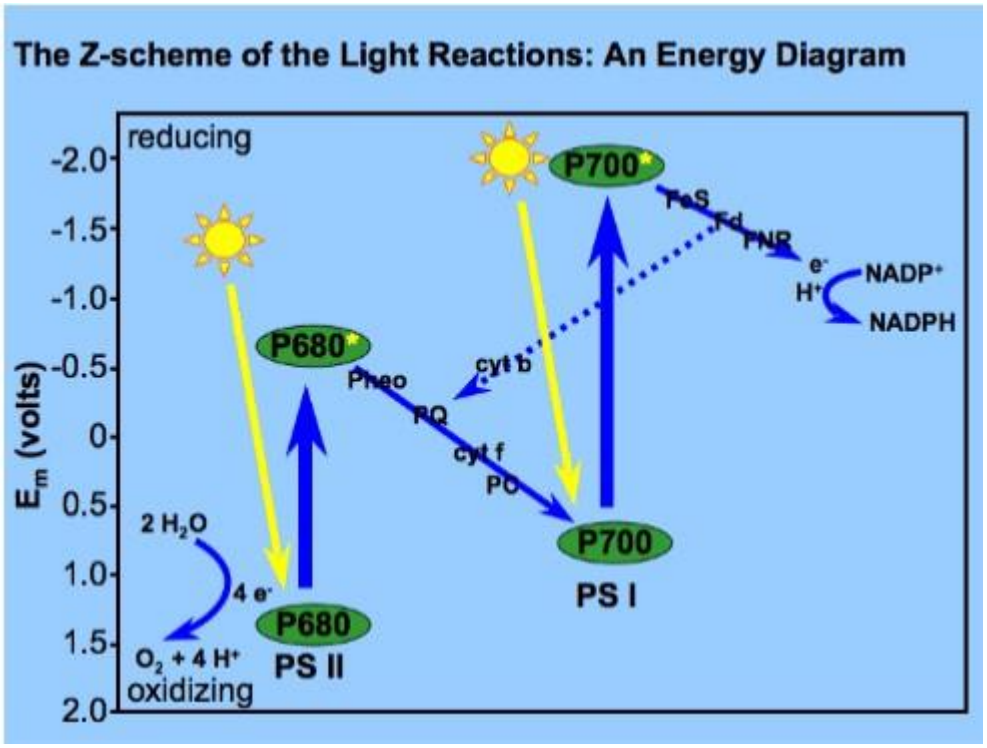

Figure 1.1 Z- Scheme of light reaction photosynthesis process (Iluz & Dubinsky, 2013)

Dark reaction otherwise called light independent reactions of photosynthesis. Carbohydrates produced from carbon dioxide by photosynthesis occur in the stroma. These reactions are indirectly dependant on light as light reactions produce ATP and NADPH. The reaction pathway is cyclic and called Calvin cycle, and it involved catalysed of enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco) with carbon dioxide into three carbon glucose phosphate molecules by reduction reactions from ATP and NADPH of the product from light reaction.

1.4 The Straits of Malacca

The Straits of Malacca, is a one of the major and most important shipping trading in the world (Ke *et al.*, 2016; Wyrtki, 1961). Narrowest part of Straits of Malacca is about 35 km wide and 30 m deep, gradually increase to about 100 m before the continental slope to the Andaman Sea begins (Wyrtki, 1961). Situated in tropical

climate, with high temperature and large amounts of rainfall throughout the year. The weather of this maritime continent is controlled by two different monsoon, the southwest monsoon from May to September, and the northeast monsoon from November to March. With both monsoon regime, it introduces variations in wind speed and direction, cloudiness, rain, and dry season over the years. The surface current in the Southeast Asian water especially in the Malacca Straits act as entry of small water exchange between Sunda Shelf and the Indian Ocean. Water movement of the Straits of Malacca is generally towards the Indian Ocean and emphatically identified with surface gradient of the sea level through these straits. Period of highest water flow in the Straits of Malacca is from January to April during the northeast monsoon. These is due to Andaman Sea experience low sea level during this period. The oceanic circulation patterns propelled by the monsoon winds play a significant role in assessing plankton distribution and other environmental parameters in the Straits of Malacca. An extensive study on variation of phytoplankton community in Straits of Malacca and Southern South China Sea by Ke *et al*. (2016) stated dominant species in this straits are include *Skeletonema, Pseudo-nitzschia, Navicula* and *Thalassionema*. In another study also conducted by Salleh *et al.* (2008), the authors reported the population in the Straits of Malacca primarily consists of two major divisions: Bacillariophyta (diatoms and Pyrrophyta (dinoflagellates). Diatoms contributed the most to the phytoplankton abundance in Straits of Malacca.

1.5 Antarctic Peninsula

Southern Ocean, also known as the Antarctic Ocean consist of the southernmost water and make up 20% of global ocean (Boyd, 2002). It is also a part of important role in controlling earth's climate. Despite of high levels of nutrient but also contribute to low level of production in open ocean water. These is due to several

factors such as mixed layer depth and advection causes by strong winds and storms (Varela *et al.,* 2002) and, shelf water where levels of phytoplankton biomass high during spring and summer. It is primarily constrained by the accessibility of iron, the main sources wherein are dust deposition and upwelling of the prevailing eastward transport of the Antarctic Circumpolar Current (ACC) (Alderkamp *et al.*, 2011). In protected coastal area such as in Antarctic Peninsula where wind and storm seldom occur, the deepening of upper mixed layer and advection processes are minimized, hence triggering the development of phytoplankton blooms (Varela *et al.*, 2002). Phytoplankton are the base of the Antarctic food web. In the Antarctic coastal water phytoplankton blooms can reach concentration approximately 10^8 cell 1^{-1} with and Chlorophyll *a* (Chl *a*) concentrations reaching as high as 50 µg 1^{-1} (Deppeler & Davidson, 2017). The phytoplankton community and its photophysiology have been extensively studied in Antarctica. Several studies have been conducted in the West of the Antarctic Peninsula (WAP) (Arrigo *et al.*, 2017; Deppeler & Davidson, 2017; Mendes *et al.*, 2017; Pereira Granja Russo *et al.*, 2017). The principle components of the planktonic community in the Southern Ocean were pigmented flagellates (prymnesiophytes, cryptophytes, prasinophytes) and nano-plankton sized diatoms groups (García *et al.,* 2013).

1.6 Problem statement

1. The study areas chosen comprised of two different climates in the coastal area. This will allow evaluation to be carried out on different ecosystem of coastal areas and understanding on the environment parameters that may affect the microalgae communities and photosynthetic activity.
2. Light regulates growth and photosynthesis in microalgae. Light limitation towards microalgae attempt will reveal the ability of this organisms to allow

flexibility to adapt to some extent. Limited study has been done especially in tropical region.

1.7 Objective

The objective of this research project are as follows:

1. To investigate and compare community structure and photo-physiological condition of natural tropical and polar microalgae communities.
2. To investigate photo-physiological responses of tropical and polar microalgae community to the effect of different irradiance exposure.

1.8 Hypothesis

1. The microalgae composition and its photophysiology in the tropical and polar region will be affected by various environmental variables.
2. Microalgae from different region (polar and tropical) will have different photo-physiological responses due to different irradiance exposure.

CHAPTER 2

LITERATURE REVIEW

2.1 Abundance and distribution of microalgae communities

Marine microalgae play an important role on earth. They made up half of the global primary production, contribute to organic matter for majority of marine organisms and crucial to the global carbon cycle (Falkowski, 2012). The community composition of microalgae blooms in Southern Ocean is dominated by diatoms, chlorophytes and phytoflagellates (Prézelin *et al.*, 2000).

The microalgae community abundance and distribution in Antarctic are widely studied. Microalgal communities have been studied in Antarctic coastal environment during austral summer in McMurdo Sound, East Antarctica (Dayton *et al*., 1986). Studies from Al-Handal & Wulff (2008) in Potter Cove, King George Island, Antarctica found 84 species of marine benthic diatoms and the common species were *Cocconeis* spp., *Gyrosigma fasciola, Navicula* cf. *cancellate, Navicula* cf. *perminuta, Petroneis plagiostoma* and *Pleurosigma obscurum*. Kang *et al.* (2002) found a variation in microalgal assemblages seasonally. The author found *Fragilaria striatula, Licmophora belgicae* and *Achnanthes groenlandica* dominated in early summer. While in winter by *Phaeocystis antarctica, Navicula glaciei* and *Navicula perminuta* dominated. This is similar with previous findings in which *Phaeocystis antarctica* dominated the regions during early spring followed by cryptophytes, diatoms and dinoflagellates (Arrigo *et al.*, 2017; Mendes *et al*., 2017). Previously in King George Island, Antarctica has observed the contribution of pelagic micro-sized diatoms in phytoplankton blooms during the growing seasons. The dominant species

found during 2010 bloom (*Porosira glacialis, Thalassiosira antarctica,* and *T. ritscheri)* are usual components of phytoplankton in Antarctica (Schloss *et al.*, 2014)

The studies of tropical marine microalgae on the abundance and distribution have been carried in Peninsular Malaysia. Based on Chua & Chong (1973), four zonal area were divided in the Straits of Malacca based on phytoplankton population: (1) low phytoplankton density on the north side due to less productive Andaman Sea; (2) medium phytoplankton density on the middle side due to upwelling from underwater that increase productivity; (3) high density of phytoplankton at the shallow water of the south side of the straits and high nutrient source from rivers from Sumatra and Malaysia Peninsular; (4) highest phytoplankton density near to the coastal. The authors reported the common diatoms presence in Straits of Malacca were *Ditylum sol, Bacillaria paradoxa, Nitzschia seriata* and *Rhizosolenia stolterforthii.* Several groups of phytoplankton were recorded at the coastal water of Manjung, Malaysia commonly Bacillariophyta followed by Chlorophyta, Cyanophyta and Dinophyta and *Odontella sinensis* are dominant species in that area (Adlan *et al.*, 2012). Naqqiuddin *et al*. (2014) compared the diversity and density of marine dinoflagellates in north and south zone of Straits of Malacca. They found out in northern zone 71 dinoflagellates species from 21 genera were found and in southern zone, 53 species form 15 genera were found with *Tripos furca* dominating both zones. *Tripos furca* was also recorded in the studies of harmful microalgae in aquaculture area in Northern Straits of Malacca (Razali *et al.*, 2015). According to Shamsudin *et al.*, (1984) the majority of the phytoplankton in the coasts of Johor, Terengganu and Kelantan comprised of *Bacteriastrum, Chaetoceros, Rhizosolenia and Pleurosigma.* Study from Salleh *et al*. (2008) recorded 50% of total population of diatoms in Pulau Perak, Pulau Sembilan and Pulau Jarak were *Bacteriastrum, Chaetoceros* and *Rhizosolenia.*

2.2 Influence of nutrient and environmental parameter on marine microalgae

Marine microalgae are capable to reproduce swiftly in which the condition is favourable for them. They can be responsive toward physical and chemical condition in the aquatic environment. Water quality of coastal water is dependent to several factors such as loads of nutrients in the system, recycling of nutrients and other combined factors within the system.

2.2.1 Nutrient

Inorganic nutrient that is essential for marine microalgae for growth and reproduction are nitrogen (ammonia or nitrate) and phosphorus (phosphate). Other inorganic nutrient such as silicate is also important, but the amount is comparatively small and its impact for marine microalgae in form of primary producer is insignificant. Marine microalgae blooms in the coastal water may be contributed from fluctuations of essential nutrient such as nitrate, phosphate and silicate from either upwelling or run-off (Suthers & Rissik, 2009). Nutrient availability is related with phytoplankton community and size composition (Lassen *et al.*, 2004). Thus, it can influence food web structure and energy flow in the pelagic ecosystem. Apart from that, nitrogen and phosphorus are used for cell membrane and protein for enzymes production. Gin *et al.* (2006) found that the growth rate size of phytoplankton elevated until optimum maximum with increased nutrient concentration along constant light intensity. This shows that phytoplankton size structure has indication on trophic state and health of water body. Nitrogen compound available in food ingredient, organic matter, fertilizer, and pesticide/herbicide can also be in natural state gases form which is colorless and odorless. Moreover, nitrogen can be found in liquid state with boiling point of 195.8°C and physically like water as it is colorless and odorless. Inorganic

nitrogen such as nitric acid (HNO_3), ammonia (NH_3), nitrogen oxide (NO, NO_2, N_2O_4, N_2O), and cyanide (CN^-). Human activity such as sewage discharge, land clearing and excessive fertilizer increase the concentration of these nutrient in the water. Altering the concentration of nutrient in natural changes the species composition of phytoplankton and some of phytoplankton may start to produce toxin (Suthers & Rissik, 2009).

2.2.2 Environmental parameter

Light is among the environmental factors that influenced photosynthesis. Microalgae have a plasticity toward light intensity in photosynthetic response (Werner, 1977). Some microalgae migrate vertically within water column to gain maximum exposure of light energy to perform photosynthesis. Marine photoautotrophs especially sea-ice microalgae face a very extreme environment with very low to high irradiances. Growth rate and carbon fixation determination is crucially as sea-ice microalgae depend on light to perform the mechanism (Petrou *et al*., 2011). Excess of light can photo-inhibit photosynthesis and it will responded to photooxidative destruction of photosynthetic apparatus (Long *et al.*, 1994).

Temperature fluctuation can bring huge impact toward seawater characteristic and living organism are dependent on it. Temperature, nutrient and light intensity may have a huge likelihood to affect the growth and migration of dinoflagellates (Naqqiuddin *et al.*, 2014). Temperature frequently affect the conveyance of organisms in shallow water and in the intertidal zone, the region that is secured at high tide and uncovered at low tide (Karleskint *et al.*, 2009). Organisms that live in this area must be able to adapt and thrive in wide range of temperature. Most of the organisms can survive in specific range of environment temperature. Above or below some critical

range of temperature may damage metabolism and decreased ability to reproduce and even death. Elevated sea surface temperature will increase rate of respiration and will decrease photosynthesis rates from light reduction (Ralph *et al.,* 2007)

Salinity is a measure of concentration of dissolved inorganic salts in water (Karleskint *et al.,* 2009). Salinity at the surface sea water are different according to latitude and topographical characteristic of an area (Karleskint *et al*., 2009). This is due to freshwater discharge from the river, evaporation, precipitation, freezing and thawing. This variation will decrease the salinity rate in surface seawater. Studies from Fakhri *et al*. (2015) suggested different amount of salinity and photoperiod rate brings significant effects toward growth of *Nannochloropsis* sp. and *Tetraselmis* sp.

2.3 Chlorophyll *a* fluorescence in marine microalgae

Fluorescence can be defined as the re-emission of photon of light with longer wavelength from the first excited state returns to the ground state (Consalvey *et al*., 2005; Huot & Babin, 2011). Light energy of red wavelength (less 670 nm) that has been absorbed excites the chlorophyll molecule from ground level to singlet state 1 (S_1). Blue light (~420 nm) has shorter wavelength and can supply enough energy to lift the electron of chlorophyll molecule to singlet state 2 (S_2). The excited electron relaxed S_2 drop to the S_1 and released heat. When the electron drops to the ground state from S_1 the energy is given off in three fates: (1) fluorescence (Huot & Babin, 2011), (2) photochemistry to drive photosynthesis (Narayan *et al.,* 2012) and (3) dissipated as heat.

Chlorophyll *a* fluorescence technique is generally utilized in study of vascular plant and extended to the investigation of microalgae and benthic biofilm due to its portable nature and rapid non-destructive measurements to the samples (Consalvey *et*

al., 2005). It is also a useful tool in photo-physiology and ecology studies (Baker, 2008). In 1966, Carl Lorenzen introduced the *in vivo* chlorophyll fluorescence technique analysis to biological oceanography field. There are a number of different fluorometers that have been developed to quantify variable chlorophyll *a* fluorescence under wide scope of conditions and for various applications.

Chlorophyll *a* fluorescence measurement in PSII has become a useful method to calculate the mechanism of photosynthesis. Pulse amplitude modulation (PAM) fluorometer expansion enable the photo-physiology measurement in terrestrial and marine plant and also widely use in field and laboratory. Kühl *et al*. (2001) conducted the first *in situ* photosynthesis measurement on sea ice algae in Greenland using Diving-PAM particularly on benthic microalgae, brown macroalgae species and coralline red algae. The usage of Diving-PAM can provide *in situ* rapid and simple analysis of the light adaptation status of phototrophic algae. Lee *et al*. (2017) conducted a study on temperature stress on *Chlorella* strain in three different region environmental samples associate with growth and photosynthesis. The author found out samples from different regions reacted differently to temperature stress with changes in photosynthetic parameter and pigment content. *Chlorella* strain from polar region experienced eurythermal adaptability with broad temperature tolerance. While *Chlorella* temperate strain was very indifferent to the temperature range measured, *Chlorella* tropical strain was already living at its upper growth temperature limit. Several photosynthetic studies has been conducted in polar region in Antarctica and Arctic region. McMinn & Hegseth (2004) carried out a study to determine the photosynthetic capacity of Arctic microalgal communities. The author found strong depth dependence on the maximum quantum yield (F_v/F_m) of Arctic microalgal communities and not showing nutrient limitation. Photoacclimation index, (E_k values)

of these communities also indicated the microalgae well adapted to the light climate. Studies conducted by García *et al*. (2013) in South Shetland Islands, Antarctica on the physiological responses of phytoplankton recorded a negative feedback on excess irradiance on photosynthetic performance at depths below 20 m. The author Phytoplankton's photosynthetic apparatus was disrupted, leading to a reduced functionality that would result to several effects, from reduced energy transfer efficiency to reduced carbon fixation ability.

Photosynthetic studies on microalgae in tropical region are still insufficient. McMinn *et al*. (2005) conducted the first photosynthetic parameter on tropical marine benthic microalgae at two different location: coastal area of Muka Head Jetty, Pulau Pinang and Pulau Songsong, Kedah. The maximum quantum yield of marine benthic microalgae produced at both locations were low using Water-PAM (McMinn *et al.*, 2005). There were significant differences between surface and bottom measurements, but the quantity was small and did not affected the photo-physiological of benthic microalgae community. Meanwhile, there were also a study on phytoplankton communities and their photo-physiological condition in Southern South China Sea and Sulu Sea by using far repetition rate fluorometry (FRRfs) (Cheah *et al*., 2013). The authors found the phytoplankton composition on the surface seawater comprised mainly of cyanobacteria, haptophytes, prochlorophytes, diatoms, and prasinophytes. produced low maximum quantum yield. The physiological conditions of phytoplankton communities of Southern South China Sea and Sulu Sea were contributed to the influence of light and nutrient concentrations (Cheah *et al*., 2013). Comparison of photosynthetic parameter using Water PAM and Pocket PAM and also nutrient limitation study in the tropical and Antarctic microalgae culture was studied by Nur Aqilah (2018). The author found inconsistencies on photosynthetic parameter

output between Water-PAM and Pocket-PAM in field and laboratory studies. Study conducted on benthic microalgae in Tanjung Rhu, Pulau Langkawi and Tanjung Bungah, Pulau Pinang from Noor Alia (2017) found that heavy metal concentrations in the environment affect the value of maximum quantum yield on benthic microalgae. The author suggested toxicity of heavy metal concentration inhibits the photophysiology processes of benthic microalgae.

2.4 Chlorophyll a fluorescence parameter

Chlorophyll fluorescence parameters enables us to evaluate the fluorescence emission from chlorophyll of PSII after the excitation by light conditions. The parameters are presented as rapid descriptor of photosynthetic processes in microalgae. Chlorophyll *a* fluorescence parameters were obtained after *in vivo* chlorophyll *a* fluorescence measurement by pulse amplitude modulated (PAM) fluorometers. List of fluorescence terminologies used are presented in Table 2.1.

Table 2.1 Fluorescence terminologies modified from (Baker, 2008; Consalvey *et al*., 2005)

	Fluorescence term	Description
Dark	F_o	Minimum fluorescence yield
	F_m	Maximum fluorescence yield during saturating flash
	F_v	Variable fluorescence
	F_v/F_m	Maximum light utilization efficiency of PS II
Light	F’	Fluorescence yield in the light-adapted state
	F_s	Fluorescence yield at steady state
	F_m’	Maximum fluorescence in the light-adapted state during saturating flash
	F_q’	Fluorescence quenched
	F_v’/F_m’	Light utilization efficiency

	F_o'	Minimum light-adapted fluorescence yield
	F_v'	Variable fluorescence in the light-adapted state
	NPQ	Non-photochemical quenching

Minimum fluorescence yield (F_o)

The yield of fluorescence is at minimal at the dark-adapted condition, F_o the reaction centre of PSII is "open" and the primary quinone acceptor of PSII (Q_A) is oxidized. F_o also can be utilized to measure biomass with two premise; the linear relationship of F_o versus Chlorophyll *a* and the light penetration must not be less than the depth of cells migrated (Consalvey *et al*., 2005; Serôdio *et al.*, 1997). Samples are kept for 15 minutes for dark adapted (Consalvey *et al.*, 2005). But, vertical migration in changes of biomass distribution has been observed in 8 minutes (Perkins *et al.*, 2002) and 15 minutes according to Consalvey *et al.* (2005). Detection of fluorescence yield beyond 15 minutes might not accurately indicate the biomass of microalgae.

Maximum quantum yield (F_v/F_m)

F_v/F_m is a test to determine the maximum quantum efficiency which light consumed by PSII used for decrease of Q_A during dark adapted (Consalvey *et al*., 2005). To carry out maximum quantum yield, the microalgae sample were placed in the dark and Q_A was turned to maximally oxidized and the reaction centers is left 'opened' (Baker, 2008). The F_v/F_m is a useful parameter as an indicator for physiological state of terrestrial plant and microalgae (Consalvey *et al*., 2005). The F_v/F_m value for non-stressed terrestrial plant is about 0.84 (Baker, 2008). Healthy microalgae is about 0.65 (McMinn & Hegseth, 2004; McMinn *et al*., 2005). Decreasing value of F_v/F_m often observed when the microalgae were stressed biotically

or abiotically. The maximum quantum yield of PSII as described by Schreiber (2004) is defined as:

$$F_v/F_m = (F_m - F_o) / F_m$$

Effective quantum yield ($F_{v'}/F_{m'}$)

During light adapted state, most of reaction centers are closed and the light utilization efficiency decreases. The microalgae exposed to saturating light pulse that maximally reduces Q_A. A prime notation (') used in fluorescence parameter shows that the sample is exposed to light that will drive photosynthesis (Baker, 2008). It happens when microalgae were induced with continuous actinic light that has a fluorescence level termed F', which rises to the maximal fluorescence level F_m'. The effective quantum yield as described by Schreiber (2004) is defined as:

$$F_v'/F_m' = (F_m'- F')/F_m'$$

Rapid light curves (RLCs)

Rapid light curves (RCLs) give definite information on the saturation characteristic of electron transport, as well as the overall photosynthetic performance of a photosynthetic organism (Ralph & Gademann, 2005). Photosynthesis-irradiance (P-E) curve is a measurement to study the photosynthetic parameters involving variety of ambient light intensities and photosynthesis. These curves are associated to the changes of photosynthetic rate and irradiance. There are three different parts in RLCs; 1) the light limited, 2) the light saturated, and 3) the photo-inhibited area. The P-E curves shows a linear, light-limited increase in photosynthetic rate and the curve level, as maximum photosynthetic capacity occurs ($rETR_{max}$). The rise of the curve, at the light limited part is termed (α), the maximum light use coefficient for PSII. Minimum saturating irradiance (E_k) is calculated by the interception of α and maximum

photosynthetic rate, ($rETR_{max}$ /α). Photoinhibition (β) indicated with higher irradiance (supra-saturating) it become inhibiting and the curves were decline and may even damaging to the photosystem complex. Relative electron transport rate (rETR) at given irradiance and E_k value described by:

$$rETR = F_v'/F_m' \times PAR$$

$$E_k = rETR_{max}/ \alpha$$

Non-photochemical quenching (NPQ)

Non-photochemical quenching (NPQ) is a process that quenches chlorophyll fluorescence and converts the excitation energy in the antenna complex to heat through non-photochemical process (Consalvey *et al.*, 2005; Goss & Lepetit, 2015). This prevent from the excitation energy from react with oxygen and become harmful reactive oxygen and provides protection from photodamaged (Narayan *et al.*, 2012).

2.5 Photoadaptation & photoprotection strategies

Light is essential in the process of photosynthesis. The rate of photosynthesis is not linearly proportional to light intensity. Thus, the reaction become limiting when it reaches saturation point. High light absorption or stressful condition exceeds the capacity of reactions in photochemistry. The reaction centre thus becomes saturated leading to photoinhibition and could damage to the photosynthetic apparatus (Petrou *et al.*, 2011). Light stresses occur slowly or quickly such as variation of sun light during the day and fluctuation of irradiance due to intertidal environment. Non-photochemical quenching (NPQ) activation is related to the short-term light stress and it dissipates electron in the form of heat. Photoinhibition is a high light condition in which the photosynthetic activity is lowered due to degradation of D1 protein for PSII (Beer *et al.*, 2014). Reaction of photoinhibition is reversible recovery, however would require

the synthesis of damaged D1 protein (Mosharov *et al.*, 2014). Microalgae experience a wide range of light environment. Ice-algae experience living under continuous low irradiance and much more subjected to photoinhibition (Long *et al.*, 1994). Photo-acclimation can be explain by the alteration of irradiance in phenotypic reaction of algae at the organism level (Falkowski & LaRoche, 1991). For examples, increased of irradiance followed with decreasing in pigment content (MacIntyre *et al.*, 2002). Meanwhile, photoadaptation is a genotypic response of algae to irradiance that has arisen during evolution (Falkowski & LaRoche, 1991). Differentiation between photo-acclimation and photoadaptation is crucial for mechanisms interpretation that control primary production in natural communities (Cullen & MacIntyre, 1998; Richardson *et al.*, 1983).

A study from Erickson *et al.*, (2015) found *Chlamydomonas reinhardtii* capable of evolving a mechanisms to manage light stress in order to avoid, minimize or repair possible damaged due to excess light. There are limited photoinhibition and photoadaptation studies on microalgae in tropical part. Study from Mosharov *et al.* (2014) concluded that the rate of illumination condition changes caused by water rise from low illuminated abyssal zone to the surface of water column are related to the photoadaptation process of phytoplankton. According to Serôdio *et al.* (2008) microalgae react to high light by enacting energy dissipating processes dependent on the xanthophyll cycle, connecting to large NPQ levels which signify a high capacity for photoprotection.

CHAPTER 3

MATERIALS AND METHODS

3.1 *In-situ* monitoring

The study locations are located at the Northern Straits of Malacca, Malaysia, and Antarctic Peninsula. The Northern Straits of Malacca comprised of five different location situated in Pulau Pinang and Kedah, Malaysia. Sampling occasions are divided into two parts: tropical region (Northern Straits of Malacca) and polar region (Antarctic Peninsula).

Northern Straits of Malacca

The Northern Straits of Malacca consisted of five sampling stations between Pulau Pinang and State of Kedah coastal area (Table 3.1) (Figure 3.1). Sampling conducted from November 2015 to February 2016.

Centre for Marine and Coastal Studies (CEMACS USM) (5.47 N, 100.20 E) located in Pulau Pinang National Park, Teluk Bahang, Pulau Pinang. Pulau Pinang National Park is known as one of the popular tourist spots in Pulau Pinang. Fishing and aquaculture activities are among the economic activity for local resident in Teluk Bahang. CEMACS USM coastal area is located in Pulau Pinang National Park where the area experience minimal disturbance from outside pollution. However, aquaculture activities located at Teluk Bahang Jetty which is not far from CEMACS USM may resulted in changes of nutrient and aquatic lives and ecology of the coastal area.

The distance from CEMACS USM to Kuala Muda station is 20.1 km. Kuala Muda coastal area (5.64N, 100.26E) is located in between Kedah and Pulau Pinang boarder. There are two large rivers flowing out: Sungai Muda and Sungai Merbok. The

main activity in both rivers are fishing activity and through the river there are local resident living along the river and plantation such as paddy field (Figure 3.1).

The distance from Kuala Muda to Pulau Bidan station is 11.1 km. Pulau Bidan (5.73N, 100.29E), Tukun Terendak (5.80N, 100.28E), & Pulau Songsong (5.81N, 100.30E) is the group of islands off the coast of Yan District, Kedah. These are uninhabited islands with the main attraction for fishing and diving activities. With the increasing popularity now, these islands have become tourist attraction for leisure and camping activities. There is a coral reef area in Tukun Terendak and Pulau Songsong. Distance from Pulau Bidan to Tukun Terendak is 7.87 km while further to Pulau Songsong station is 2.25 km.

Table 3.1 Sampling location of tropical region in Northern Straits of Malacca

Location	**Sampling area**
CEMACS USM	5.47 N, 100.20 E
Kuala Muda	5.64 N, 100.26 E
Pulau Bidan	5.73 N, 100.29 E
Tukun Terendak	5.80 N, 100.28 E
Pulau Songsong	5.81 N, 100.30 E

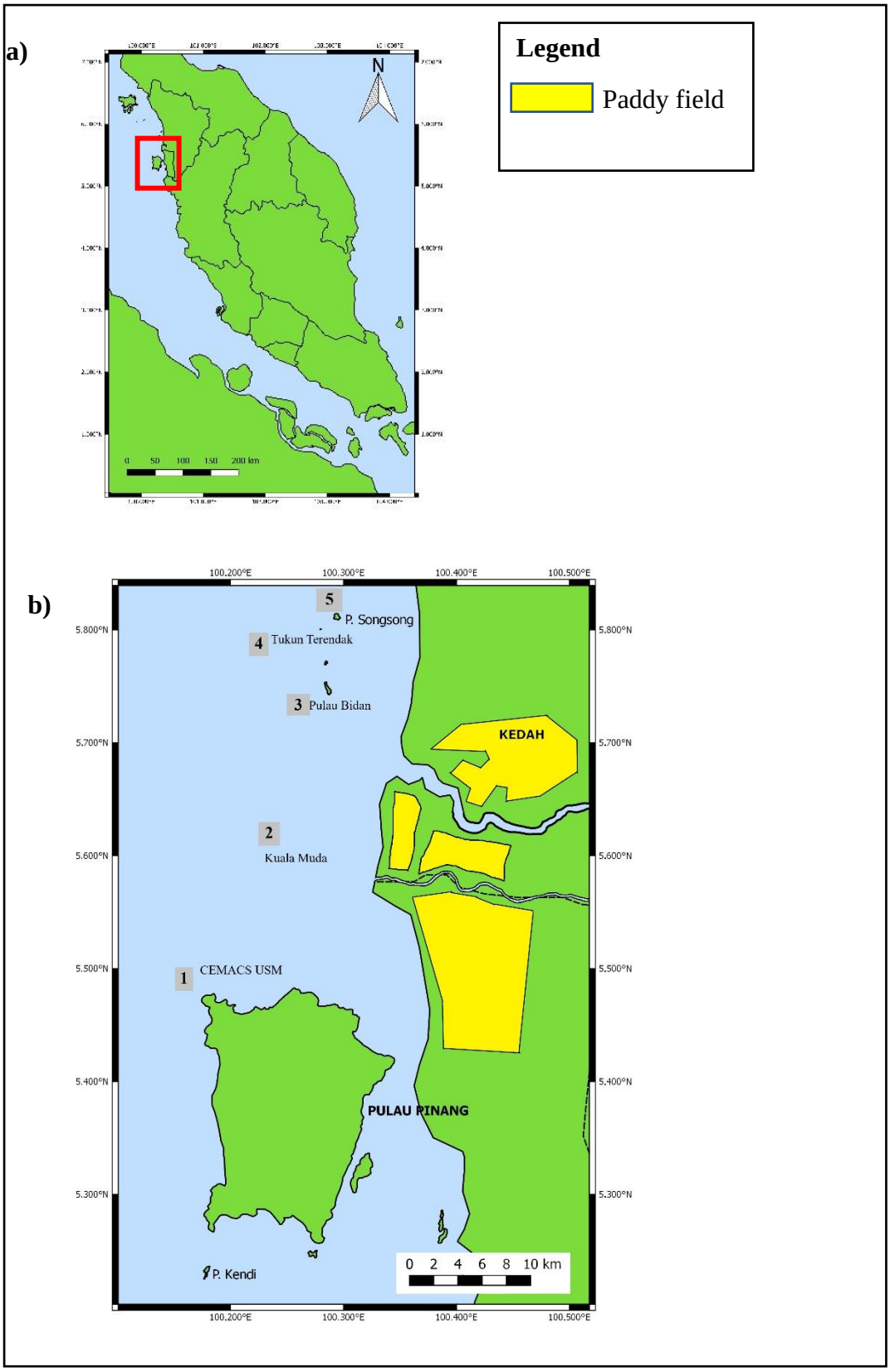

Figure 3.1 Map of Peninsular Malaysia showing Pulau Pinang and State of Kedah. a) Map of Peninsular Malaysia. b) Map of Pulau Pinang and State of Kedah. Numbering indicate the sampling location. 1) CEMACS USM, 2) Kuala Muda, 3) Pulau Bidan, 4) Tukun Terendak and 5) Pulau Songsong.