PHOTOSYNTHETIC CAPABILITIES AND RESPONSES OF HARD CORALS TO TURBIDITY INFLUENCE AND THERMAL STRESS

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

±	plus minus
%	percentage
°C	degree Celcius
cm^2	centimetre per squared
km	kilometre
L	litre
m	metre
mg	milligram
mg/L	milligram per litre
mL	milliliter
ppt	parts per thousand
r	Pearson's correlation coefficient
$\mu mol\ m^{-2}\ s^{-1}$	micromole per metre per squared per second
α	photosynthetic efficiency
β	photoinhibition
ANOVA	analysis of variance
ATP	adenosine triphosphate
$CaCO_3$	calcium carbonate
CO_2	carbon dioxide
CEMACS	Centre for Marine and Coastal Studies
D.O	dissolved oxygen
E_k	photoacclimation index
F_o	minimum fluorescence yield
F_m	maximum fluorescence yield during the saturating flash

F_v	variable fluorescence
Ft	fluorescence yield in the light adapted state
$F_{m'}$	fluorescence yield in the light adapted state during the saturating flash
$F_{v'}$	fluorescence quenched
F_v/F_m	maximum quantum yield of PSII
$F_{v'}/F_{m'}$	effective quantum yield
H_20	water
HCl	hydrochloric acid
NH_3	ammonium
NO_3^-	nitrate
NO_2^-	nitrite
rETR	relative electron transport rate
rETRmax	maximum relative transport rate
NPQ	non-photochemical quenching
PAM	pulse-amplitude modulation
PAR	photosynthetically active radiation
PO_4^{2-}	phosphate
PSI	Photosystem I
PSII	Photosystem II
qP	photochemical quenching
RLC	rapid light curve
RuBisCo	Ribulose-1, 5-biphosphate carboxylase/oxygenase
SPSS	Statistical Package for Social Science
SST	sea surface temperature

TSS	total suspended solids

KEBOLEHUPAYAAN DAN GERAK BALAS FOTOSINTESIS KARANG TERHADAP PENGARUH TURBIDITI DAN TEKANAN SUHU

ABSTRAK

Ianya telah dihipotesiskan bahawa spesies dan koloni batu karang yang berlainan mempunyai tindak balas foto-fisiologi yang berbeza terhadap pendedahan 2 jam dan 24 jam dan peningkatan suhu disebabkan penyesuaian "zooxanthellae" terhadap persekitaran semulajadi. Tesis ini mengkaji kesan tempoh pendedahan dan peningkatan suhu air laut pada foto-fisiologi karang keras dari Pulau Kendi, Pulau Pinang dan Pulau Songsong, Kedah. Pulau Kendi dan Pulau Songsong terletak di utara Semenanjung Malaysia, kedua-duanya dikenali sebagai kawasan pemendapan yang tinggi dan keadaan cahaya rendah dengan radiasi aktif fotosintetik 24.707 ± 1.329 µmol m^{-2} s^{-1} dan 54.267 ± 1.739 µmol m^{-2} s^{-1} di Pulau Kendi dan Pulau Songsong (diukur pada 12pm). Untuk mengkaji foto-fisiologi karang dalam air keruh Pulau Kendi dan Pulau Songsong, satu kajian *in-situ* menggunakan fluorometer "DIVING-Pulse-Amplitude Modulated (PAM)" telah dijalankan. Nilai hasil kuantum maksimum (F_v/F_m) yang tertinggi telah dicatat oleh spesies *Porites lutea* (0.787 ± 0.034) dan *Goniastrea aspera* (0.730 ± 0.010) dari Pulau Kendi and Pulau Songsong. "Rapid light curve (RLC)" yang diperolehi menunjukkan trend yang sama untuk semua spesies dengan saturasi cahaya berlaku pada2000.00 µmol m^{-2} s^{-1}. Parameter fotosintesis (kadar pengangkutan elektron maksimum (rETRmax), kecekapan fotosintesis (α) dan indeks penyesuaian cahaya (E_k) diperolehi menunjukkan bahawa semua spesies mempunyai aktiviti fotosintesis yang berbeza walaupun mempunyai morfologi yang sama. *Turbinaria mesenterina* menunjukkan nilai α yang

tertinggi menunjukkan bahawa karang jenis plat mempunyai kadar fotosintesis yang lebih baik berbanding spesies lain. Empat spesies karang keras diambil dari kedua-dua lokasi untuk menyiasat tindak balas fisiologi cahaya apabila terdedah kepada masa pendedahan yang berbeza dan peningkatan suhu. Suhu air dinaikkan sebanyak 2 °C dari 31-37 °C selama 2 jam (jangka pendek) dan 24 jam (jangka panjang). Semua parameter fotosintesis seperti F_v / F_m, F_v '/ $F_{m'}$, "photochemical quenching" (qP) dan "non-photochemical quenching" (NPQ) berkurangan apabila suhu meningkat. Spesies yang paling tahan berdaya tahan dalam tekanan peningkatan suhu adalah *Porites lutea* dari Pulau Kendi dan *Turbinaria mesenterina* dari Pulau Songsong Jumlah tenaga cahaya yang hilang sebagai haba (NPQ) meningkat apabila suhu meningkat sehingga 33°C. Nilai NPQ yang meningkat adalah konsisten dengan penurunan nilai F_v/F_m di dalam peningkatan suhu menunjukkan bahawa NPQ telah diaktifkan sebagai pelindung cahaya. Kajian ini mencadangkan bahawa spesies karang yang berbeza mempunyai fisiologi cahaya yang berbeza apabila terdedah kepada masa pendedahan yang lebih panjang dan pendek. Prestasi fotosintesis berkurangan apabila suhu meningkat disebabkan komponen fotosintesis yang rosak.

PHOTOSYNTHETIC CAPABILITIES AND RESPONSES OF HARD CORALS TO TURBIDITY INFLUENCE AND THERMAL STRESS

ABSTRACT

It was hypothesized that different species and colonies of corals have different photo-physiological responses to exposure time and elevated temperature. This thesis investigates the effect of 2 hours and 24 hours exposure and elevated seawater temperature on the photo-physiology of hard corals collected from Pulau Kendi, Penang and Pulau Songsong, Kedah. Pulau Kendi and Pulau Songsong are located at the northern region of Peninsular Malaysia, both are known as highly sedimented areas with low-light condition with photosynthetically active radiation (PAR) values of 24.707 ± 1.329 µmol m^{-2} $s^{-1}$and 54.267 ± 1.739 µmol m^{-2} s^{-1} respectively (measured at 12pm). To investigate the photo-physiology of corals in turbid water from both study sites, an *in-situ* photo-physiology measurements were conducted using DIVING-Pulse Amplitude Modulated (PAM) fluorometer. Maximum quantum yield (F_v/F_m) values were recorded the highest was found in *Porites lutea* (0.787 ± 0.034) and *Goniastrea aspera* (0.730 ± 0.010) from Pulau Kendi and Pulau Songsong respectively. Rapid light curves (RLCs) showed similar trend for all species with light saturation occurring at 2000.00 µmol m^{-2} s^{-1}. Photosynthetic parameters obtained showed that all species have different photosynthetic activity despite having similar morphology. *Turbinaria mesenterina* showed the highest α (0.040 ± 0.002) which indicated that plate type coral has better photosynthetic efficiency compared to other morphology type. Four hard corals species were collected from both sites to investigate the photo-physiological responses when exposed to different exposure time and increasing

temperature. Corals were exposed to increasing water temperature from 31-37°C for 2 hours (short term) and 24 hours (long term). All photosynthetic parameters (F_v/F_m, $F_{v'}/F_{m'}$, rETRmax, α, E_k and non-photochemical quenching (NPQ) decreases at temperature 35°C. Most resilient species to elevated temperature stress were *Porites lutea* from Pulau Kendi and *Turbinaria mesenterina* from Pulau Songsong. The amount of light energy dissipated as heat (NPQ) increased as temperature increased up to 33°C. Increased values of NPQ was also consistent with decreased F_v/F_m values in increasing temperature indicating that NPQ is activated for photoprotection. This study suggested that different coral species have different photo-physiology when exposed to longer and shorter exposure time. Photosynthetic performances of corals were reduced as temperature increased due to the damage photosynthetic components.

CHAPTER 1

INTRODUCTION

1.1 Hard corals

Hard corals are coral species from Order Scleractinia of Phylum Cnidaria. All hard corals are made of a crystallized form of calcium carbonate ($CaCO_3$) known as aragonite skeleton (Barnes, 1987). They build coral reefs by depositing the calcium carbonate skeleton to form the stony framework of the reef (Ladd, 1961). Hard corals can be found in colonies and are composed of hundred thousand of individuals, called polyps (Barnes, 1987; Lalli & Parsons, 1995). In hard corals, the polyp secretes a small limestone cup which is also called a corallite and function as protection for the soft polyp tissue (Ladd, 1961). The annual growth of corals is very slow as it may increase in size from a few mm to 5cm every year depending on the environment (Karuppanapandian & Karuppudurai, 2007).

Most of hard corals contain single-celled photosynthetic algae which live within their tissue known as zooxanthellae. Symbiotic relationship between these organisms is the key factor in coral reef productivity thus ensuring the survival of coral colonies in their habitat (Appeldoorn *et al*., 2009). The role of zooxanthellae is mainly providing nutrients to their coral hosts. In return, the coral provides protected environment and compounds necessary for photosynthesis (Appeldoorn *et al*., 2009). Zooxanthellae are expelled by coral tissues due to increase in temperature and limited light penetration. When this happens, corals will turn whitish or bleach which is commonly described as "coral bleaching" (Barnes & Hughes, 1999; Lalli & Parsons, 1995).

Coral reefs are the centre of high biodiversity which are home for million species of marine organisms making it the most diverse ecosystem on earth. They play important ecological roles by providing refuge, habitat, breeding and nursery ground for large number of marine organisms including molluscs, fishes and others (Hoegh-Guldberg *et al*., 2007). In addition, coral reefs also serve as natural breakwaters, protecting shorelines from wave erosion, while creating lagoons. Coral reefs are distributed along the tropical and subtropical region. Wilkinson (2008) stated that most of coral reefs are distributed along the tropical and subtropical waters in Middle East, Asia, Pacific and Australia, West Caribbean countries. Coral reefs are found in these area due to their requirements for growth which the water must be shallow and clear (Wilkinson & Buddemeier, 1994). In addition, tropical coral can live and grow within 18-30°C. Water temperature outside the range of 18-30°C will result in loss of zooxanthellae from coral tissue (Spalding *et al*., 2001).

1.2 Hard corals of Malaysia

Over 30% of the world's coral reefs are located in the Coral Triangle (Yasin, 2011; Buddumeir *et al*. 2004). Malaysia is a part of "Coral Triangle", a marine area located in western Pacific Ocean which include the waters of Indonesia, Malaysia, the Philippines, Papua New Guinea, Timor Leste and Solomon Island. It is recognized by scientists and researchers as the world's richest marine biodiversity. Highest diversity of corals can be found in Malaysia whereby over 550 species was found in East Malaysia and over 480 species in Peninsular Malaysia. The total coastline for Malaysia is 4800 km with 2100 km for Peninsular Malaysia and 2700 km for East Malaysia (Burke *et al*., 2001). Extensive coral reef survey in Malaysia was recorded since 1977 but much of these are unreported

and dispersed among institution (Tun *et al*., 2004). A study by Toda *et al*. (2007) in Peninsular Malaysia proved that 17.9% to 68.6% is covered by living corals. A total coral coverage community of 74.5% are dominated by Genus *Acropora*, Genus *Porites*, and Genus *Montipora* while 2% and 5% are occupied by Genus *Goniastrea*, Genus *Heliopora*, Genus *Galaxea* and Genus *Pavona*. According to Chou (1998), coral reefs are richer on east coast than west coast around offshore islands in Peninsular Malaysia. Corals reefs on Peninsular Malaysia are along eastern coast and offshore islands and west coast. Corals reefs fringe the northern offshore islands of Pulau Langkawi, Pulau Payar and Pulau Perak in the state of Kedah and Pulau Pangkor, Pulau Jarak and Pulau Sembilan in the state of Perak. Small, poorly developed and heavily degraded coral reefs occur in the southern state of Negeri Sembilan, at Port Dickson and Tanjung Tuan. Extensive fringing coral reefs are also found in Sabah, East Malaysia also associated with offshore islands away from southeast coast. Coral reefs in Sipadan were found to be in the best condition in Sabah. In Sarawak, most abundant corals are found in coast off Miri primarily offshore Luconia reefs (Ridzwan & Cabanban 1994).

1.3 Effect of climate change on coral ecosystem

Coral reefs have been degrading since past few decades and are at risk because of natural and anthropogenic stressors. Increase in sea water temperature as a result of climate change is one of the most serious natural stressor to corals throughout the world (Mouchka *et al*., 2010; Ruiz-Morenol *et al*., 2012). Abnormally higher sea water temperatures (1-2°C higher than ambient temperature) will cause coral bleaching. (Glynn, 1993; Hoegh-Guldberg *et al*., 2007). Coral bleaching is the potentially lethal condition where corals become white due to heat stress. This condition induced the reduction in

concentration of their zooxanthellae which are photosynthetic dinoflagellate symbionts live within coral tissues (Brown *et al.*, 1995). Prolonged condition of this provide adverse condition for zooxanthellae will eventually cause the zooxanthellae to leave coral tissues (Hoegh-Guldberg *et al.*, 2007). High temperature will damage the photosynthetic components in zooxanthellae. If the environmental condition does not return to normal, zooxanthellae will not return to corals and this will cause them to die due to insufficient nutrient to live (Hoegh-Guldberg *et al.*, 2007). In addition, coral bleaching can result in coral mortality, declines in coral percentage cover and shifts in the population of other reef-dwelling organisms (Pratchett *et al*., 2008). If the thermal stress decreases, corals may recover, but if the stress is prolonged, mortality may occur. Outbreaks of coral disease typically occurs after bleaching events since stressed corals are more susceptible to infection (Randall *et. al*, 2014).

Ocean acidification can also harm coral reefs as sea water becomes more acidic when more excess carbon dioxide (CO2) in the atmosphere dissolved into the ocean. According to Sabine *et al*. (2004), oceans have absorbed approximately 1/3 of the carbon dioxide produced from human activities and 1/2 of the carbon dioxide produced by burning fossil fuels since year 1800. Ocean acidification makes it difficult for corals to absorb and maintain calcium carbonate in their skeletons. The stony skeletons that support the coral reefs will dissolve and be weaken, making it more vulnerable to disease and destruction by natural disasters such as storms.

1.4 Coral photosynthesis

Photosynthesis is a process by which light plants, algae, some bacteria and some protists convert light energy to produce glucose from carbon dioxide and water. In this process, carbon from carbon dioxide is fixed into organic carbohydrates, using reducing agents derived from the splitting of water to release oxygen. The glucose produced can be converted into pyruvate which releases adenosine triphosphate (ATP) by cellular respiration. Oxygen is also formed. Photosynthesis may be summarised by the word equation:

$$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O \text{ (Van Niel, 1992)}$$

Photosynthesis occurs in the chloroplast of photosynthetic organisms. The process of photosynthesis can be divided into two sets of reactions: (1) the light reactions, which H_2O is split (occurs in the thylakoid membrane) and (2) dark reactions, which is a carbon reduction reaction (occurs in the stroma) (Bassham *et al*., 1950). Light absorption is first phase that occur in photosynthesis. Photopigments are molecules that play important roles in absorbing light energy. Chlorophyll *a* is the ubiquitous pigment which can be found in all eukaryotic plants, algae and prokaryotic cyanobacteria. It is a main pigment which absorbs red (650-700nm) and blue (400-450nm) bands of the spectrum. Several modifications of chlorophyll occur among plants and other photosynthetic organisms. For example, algal cells and higher plants have accessory pigments such as fucoxanthin which can increase the range of wavelengths that can be used for photosynthesis. Carotenes and xanthophylls are carotenoid pigments involved in energy transfer which can protect cells from damaging reactive singlet oxygen (Sandmann *et al*., 1983).

1.5 Problem statements

Turbidity caused by particles suspended or dissolved in water that scatter light making the water appear cloudy or murky. Particulate matter can include sediment - especially clay and silt, fine organic and inorganic matter, soluble colored organic compounds, algae, and other microscopic organisms. Turbidity is one of stressor that cause serious problem to marine ecosystem especially the coral reef ecosystems. Coastal reclamation and construction along of Penang and Kedah coastal areas which is also near to study sites may cause high water turbidity to nearby marine ecosystems. Turbidity occurs when eroded material that is being transported by water, settles out of the water column onto the water surface, as the water flow slows. Consequently, water turbidity increases causing less light penetration to the sea floor.

Elevated temperature is another stressor that has been identified as a major factor affecting coral health. Hard corals can only survive up to 1-2°C above ambient temperature (Glynn, 1993). Malaysia experiences wet and dry season every year. During the dry season, sea surface temperature (SST) could increase up to 3 to 4°C higher than usual temperature which will also increase sea water temperature. Temperature influences algal photosynthesis by changing photosynthesis rates, or by inducing phenotypic or genotypic changes (Davison, 1991). Increase in seawater temperature has the potential to alter the biomass and species composition of benthic microalgal communities (Defew *et al*., 2004).

The introduction of submersible Diving-PAM fluorometer enables the study of photosynthetic performance of corals. Chlorophyll *a* fluorescence analysis enables researchers to study the dissipation of absorbed excitation energy by PSII (Falkowski &

Raven, 1997). Apart from that, the use of PAM enables the study of PSII electron transport rate (ETR) that is produced by photosynthesis through rapid light curves. Rapid light curves (RLCs) allow the characterization of the acclimation status to recent light history, to be obtained in a short time prior to the start of the curve (Schreiber *et al*., 1997; Ralph *et al*., 1999; White & Critchley, 1999; Serôdio *et al*., 2005). Quenching analysis also enables the study of how coral protective mechanisms work by dissipating excess excitation energy to heat.

This thesis investigates the photo-physiology of different hard coral species in turbid water and low-light condition of Pulau Kendi and Pulau Songsong. Both sites share the same condition which is known to be turbid water areas (Chua *et al.*, 2000). Therefore, the light penetration was low for both sites. At Pulau Songsong, coral reefs could be found mainly on the southern part of the island whereas smaller colonies of corals may be found elsewhere (Norhanis *et al*., 2012). Live coral cover recorded during the study was 16.8%. Meanwhile, there is no published work at Pulau Kendi therefore, live coral cover was not known. During the study, coral reefs were found at the southern part of Pulau Kendi. Only a few studies have been done in Pulau Songsong but none of them was related to coral photo-physiological study. This thesis also investigates the effect of short-term and long-term exposure of increasing temperature on different hard coral species from both Pulau Kendi and Pulau Songsong using Diving-PAM. Temperature-based experiments were done onto four coral species. *Turbinaria mesenterina*, *Goniastrea minuta*, *Porites lutea* and *Goniopora cellulosa* were collected from both sites and were maintained in Centre for Marine and Coastal Studies (CEMACS) prior to the experiment. A preliminary water

quality assessment was done in CEMACS to determine the suitability of filtered sea water in CEMACS culture facility for experimental use.

1.6 Objectives

The objectives of this study are as follows:

1. To investigate the photoadaptation of different species of hard corals in turbid water of Pulau Kendi and Pulau Songsong.
2. To determine the photo-physiology of different species of hard corals of Pulau Kendi and Pulau Songsong towards 2 hours and 24 hours elevated temperature exposure.

1.7 Hypotheses

The hypotheses tested in this study are:

1. Hard corals are able to adapt to turbid water condition of Pulau Kendi and Pulau Songsong
2. Different coral species have different photo-physiological responses to different exposure and elevating temperature.

CHAPTER 2

LITERATURE REVIEW

2.1 Chlorophyll fluorescence

Chlorophyll fluorescence parameters allow us to quantify the fluorescence emissions from chlorophyll of PSII after excitation by light conditions. The parameters are presented as rapid descriptors of photosynthetic processes in microalgae. Chlorophyll fluorescence parameters are obtained after *in vivo* chlorophyll fluorescence measurements by pulse amplitude modulated (PAM) fluorometers.

Quantum yield efficiency (F_v/F_m and F_v'/F_m') of PSII

F_v/F_m is a good indicator of maximum quantum yield of PSII chemistry (Butler, 1978; Genty *et al*., 1992) and photoinhibition of microalgae (Bergmann *et al*., 2002; Consalvey *et al*., 2005; Cavender-Bares & Bazzaz, 2004). To achieve maximum quantum yield, samples are kept in the dark in which the reaction centres are said to be "opened" (Baker, 2008). These cells are relaxed during dark adaptation period. The F_v/F_m value of healthy microalgae is between 0.6 and 0.7 (Kromkamp & Peene, 1999; McMinn & Hegseth, 2004; White *et al*., 2011), usually less than that of vascular plants. However, this depends on the type of fluorometer used as well. The decrease of F_v/F_m is often observed when microalgae are exposed to harsh conditions.

Effective quantum yield (Fv'/Fm')

In light-adapted state, the effective quantum yield denoted by $F_{v'}/F_{m'}$ is used to determine the PSII operating efficiency under different light and other environmental

conditions (Baker, 2008; Genty *et al*., 1989). A prime notation (') indicates sample is exposed to light (e.g. natural light and actinic light) that will drive photosynthesis. When actinic light is introduced in microalgae, the fluorescence is termed as F' which rises to maximum fluorescence, $F_{m'}$.

Rapid light curves (RLCs)

Rapid light curves (RLCs) provide detailed information on the saturation characteristics of electron transport, as well as the overall photosynthetic performance of photosynthetic organism. The RLCs, in which mass-specific photosynthetic rates are plotted versus irradiance, is commonly used to characterize photo-acclimation. RLCs can also provide an assessment of photosynthetic activity, by integrating the algae's ability to tolerate light fluctuation, as well as reflecting its immediate short-term light history (Schreiber *et al*., 1997; White & Critchley, 1999). The photosynthetic parameters, maximum relative transport rate (rETRmax), photosynthetic efficiency (α) and photoacclimation index (E_k) are estimated from RLCs by fitting the relative electron transport rate (rETR) versus E (irradiance) data to an exponential curve. There are three distinct part in RLC; 1) the light limited, 2) the light saturated and 3) the photo-inhibited part. RLC show a linear, light limited increase in photosynthetic rate until it reaches a maximum light saturated rate (rETRmax). The rise of the curve, which is the light limited part of the curve is termed (α), the maximum light use coefficient for PSII. The light saturation coefficient (E_k) is calculated as rETRmax/α. Photoinhibition (β) occur above the irradiance at which the photosynthetic rate saturates. At this level, excess light may be even damaging to the photosystem complexes.

Photochemical (qP) and non-photochemical quenching (NPQ)

Excess photon energy in PSII can either drive photosynthesis (photochemical quenching, qP) converted to heat (nonphotochemical quenching, qN and NPQ). Photochemical quenching (qP) transport the electrons that cannot be used in PSII away from PSII due mainly to the light-induced activation of enzymes involved in carbon metabolism and opening of stomata (Kautsky & Amman, 1960). Heat dissipation is linked to the xanthophyll cycle, which protects the photosynthetic apparatus from high-light damage.

NPQ can be used to infer activity of the xanthophyll cycle (Demmig-Adams & Adams, 1992; Ralph *et al*., 2005). Quenching analysis compares the fluorescence yield during a saturating pulse under actinic light conditions ($F_{m'}$ and F), with the dark-adapted values (F_m and F_o). Non-photochemical quenching and $F_{v'}/F_{m'}$ are correlated, where $F_{v'}/F_{m'}$ decreases with increasing irradiance, as more electrons accumulate at the PSII acceptor side and there is a relative increase in NPQ (Schreiber, 2004). The NPQ process has been shown to increase protection from high light by dissipating excess energy as heat in the PSII. This process linked to the operation of the xanthophyll cycle (Falkowski & Raven, 1997; Muller *et al*., 2001; Van Leeuwe & Stefels, 2007; Van Leuwee *et al*., 2008).

2.2 Effect of light on the photosynthesis of corals

Light is one of the most important requirements in photosynthesis (Falkowski & Raven, 1997; Kirk, 1994; Ting & Owens, 1994; Longhi *et al*., 2003). Many studies have provided information on how algae responses to different range of light in their natural environment. Although light is crucial for all photosynthetic activity, excess light may

lead to increased production of damaging reactive oxygen (Neori *et al*., 1984; Muller *et al*., 2001). As a result, photoinhibition will occur which will lead to damage to Photosystem II (PSII) (Honeywill *et al*., 2002). Photoinhibition can cause a reduction in the maximum quantum yield (F_v/F_m) as a result of damaged PSII. NPQ will be activated to protect the cell from excess light. According to Winters *et al*. (2003), *Stylophora pistillata* (branching coral) showed decreased rETRs and increased NPQ in the afternoon compared to morning hours. The decreased of rETR and NPQ values in the afternoon indicated reduced photosynthetic capacity due to photoinhibition. Non-photochemical quenching is activated at high light to protect the cell from excess light. Dissipation of excess excitation energy by the xanthophyll cycle is widely recognised as an important photoprotective mechanism (Arsalane *et al*., 1994). The ability of chlorophyll to deactivate excited PSII by the conversion of the xanthophylls diadinoxanthin to diatoxanthin is well documented in Young & Frank, (1996). Xanthophyll cycle will be activated which the pigment diadinoxanthin (DD) is rapidly converted to energy dissipating pigment (DT). A study by Brown *et al*., (1999) on the xanthophylls DD and DT showed a strong inverse correlations between the xanthophyll ratio DD/(Dn + DT) and F_v/F_m and $F_{v'}/F_{m'}$. Apart from that, few studies proved that photoinhibition can be reduced in zooxanthellae by adjusting various cellular components including pigmentation and number of 'reaction centres', a light harvesting component of chlorophyll (Falkowski & Dubinsky, 1981; Iglesias-Prieto & Trench, 1994; Robison & Warner, 2006).

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be used to infer activity of the xanthophyll cycle (Demmig-Adams & Adams, 1992; Ralph *et al*., 2005). Quenching analysis compares the fluorescence yield during a saturating pulse under actinic light conditions ($F_{m'}$ and F), with the dark-adapted values (F_m and F_o). In high irradiance, NPQ was activated as a mean of photoprotective mechanism to prevent damage to PSII during photoinhibition (Muller *et al*., 2001). It helps to regulate and protect photosynthesis in environments in which light energy absorption exceeds the capacity for light utilization. However, RLCs trend for all species from both sites were similar with the light saturation occuring at 2000 μmol m^{-2} s^{-1} showing that all species from both sites have the same light limitation level. Non-photochemical quenching and $F_{v'}/F_{m'}$ are correlated, where $F_{v'}/F_{m'}$ decreases with increasing irradiance, as more electrons accumulate at the PSII acceptor side and there is a relative increase in NPQ (Schreiber, 2004). The NPQ process has been shown to increase protection from high light by dissipating excess energy as heat in the PSII. This process linked to the operation of the xanthophyll cycle (Falkowski & Raven, 1997; Muller *et al*., 2001; Van Leeuwe & Stefels, 2007; Van Leuwee *et al*., 2008).

2.3 Effect of temperature on the photosynthesis of corals

Increased SST is a major factor triggering coral bleaching and decreasing photosynthetic efficiency of symbiotic dinoflagellates (Hoegh-Guldberg, 1999). High temperature stress mainly damages the functional disorders of photosynthetic apparatus in PSII (El-Sabaawi & Harrison, 2006). A reduction in F_v/F_m and $F_{v'}/F_{m'}$ values are the results of damaged photosynthetic apparatus in photosynthetic organisms. Temperature has a major effect on photosynthetic parameters because it is controlled by enzyme reactions (Davison, 1991; Underwood & Kromkamp, 1999). Temperature-induced coral

bleaching results from the impairment of PSII function, due to accumulated light-dependent damage to protein (D1) which can be found in PSII reaction centres (Warner *et al*., 1999; Takahashi *et al*., 2009; Hill *et al.*, 2011). Several other components were also sensitive to temperature and may contribute to the onset of bleaching or enhance the effects of lower PSII function once bleaching begins. For example, high temperatures can inactivate Rubisco (Lilley *et al*., 2010) and interrupts the reactions of Calvin Cycle (Jones *et al*., 1998). However, monitoring of PSII photoinhibition by Takahashi *et al.*, (2004) revealed that heat dependent photoinhibition was ascribed to inhibition of the repair of photodamaged PSII. The efficiency of the photosynthesis repair machinery determines the bleaching susceptibility of coral species under elevated seawater temperature. Hoogenboom, (2012) showed similar findings which PSII and whole chain electron transport of *Stylophora pistillata* was susceptible to temperature stress due to photoinibitory repair mechanism. PSII photoinhibition by excessive temperature is a well-known cause of the reduction of photosystem (PSII) quantum efficiency and electron transport carrier (Coles & Jokiel, 1978; Beer *et al*., 1998; Franklin *et al*., 2006; Chow *et al*., 2009).

To maintain an optimal photosynthesis during thermal stress, algal cells have evolved mechanisms referred to as photoprotection which optimize photosynthetic efficiency (Perkins *et al*., 2006). At low light levels, these mechanisms ensure that maximum photosynthetic efficiency rates are maintained, whilst at high light level, they prevent photodamage or photoinhibition (Muller *et al*., 2001). According to Muller *et al*. (2001), non-photochemical quenching (NPQ) is often activated to protect microalgae from excess energy. During this process, the xanthophyll cycle is activated and the pigment DD

is rapidly and reversibly converted to the energy dissipating pigment DT (Casper-Lindley & Björkman, 1998; Serôdio *et al*., 2005). If NPQ is inactivated due to light or temperature stress, the excess energy can damage the PSII and induce photoinhibition. NPQ of chlorophyll fluorescence is an indicator of the level of excess energy dissipation in the light-harvesting antennae of PSII (Maxwell & Johnson, 2000). Middlebrook *et al*. (2008) stated that corals developed a photoprotection mechanism which is non-photochemical quenching when exposed to increasing temperature. Increasing temperature also reduces 40% of coral symbionts (zooxanthellae) causing a reduction in photosynthetic efficiency.

Thermal stress is responsible for decreasing photosynthetic rates at high and low temperatures, and also reduces the efficiency of the photosynthetic electron transport carrier (Anning *et al*., 2001; El-Sabaawi & Harrison, 2006). High temperature stress reduces variable fluorescence (F_v), maximum quantum yield (F_v/F_m) and the effective quantum yield ($F_{v'}/F_{m'}$) indicating that structural and functional disorders of the photosynthetic apparatus occur if PSII is damaged (El-Sabaawi & Harrison, 2006). This was supported in a study by Rodrigues *et al*., (2008), which F_v, F_m, F_v and F_v/F_m values of corals decreased to 90%, 87% and 83% after exposed to higher temperature. Temperature has a major effect on photosynthetic parameters, particularly electron transport rate (ETR), because it is controlled by enzyme reactions (Underwood & Krompkamp, 1999).

In hard corals, several studies have shown that increasing temperature decreased the photosynthetic efficiency of the symbiotic zooxanthellae and most often lead to coral bleaching events (Fitt *et al*., 2001; Bhagooli & Hidaka, 2004; Hill *et al*., 2004). Similar finding was obtained by Ferrier-Pagès *et al*. (2007) which stated that increasing

temperature to 34°C significantly decreased the F_v/F_m in *Stylophora pistillata* and *Montipora aequituberculata*. A study by Rodolfo-Metalpa *et al*. (2006) also stated that rETRmax and F_v/F_m values decreased in increasing temperature. F_v/F_m of *Oculina patagonica* decreased by 9% at 32°C after 5h incubation. In addition, it was observed that NPQ values increased in increasing temperature indicating activated photoprotection against temperature stress. However, there are less studies comparing short-term and long-term exposure effects on corals in increasing temperature.

CHAPTER 3

MATERIALS AND METHODS

This study focuses on two parts which are (i) *in-situ* photo-physiology measurements of hard corals from Pulau Kendi and Pulau Songsong and (ii) effect of exposure time and increasing temperature on the photo-physiology of hard corals from Pulau Kendi and Pulau Songsong. Methodologies are summarized in (Figure 3.1).

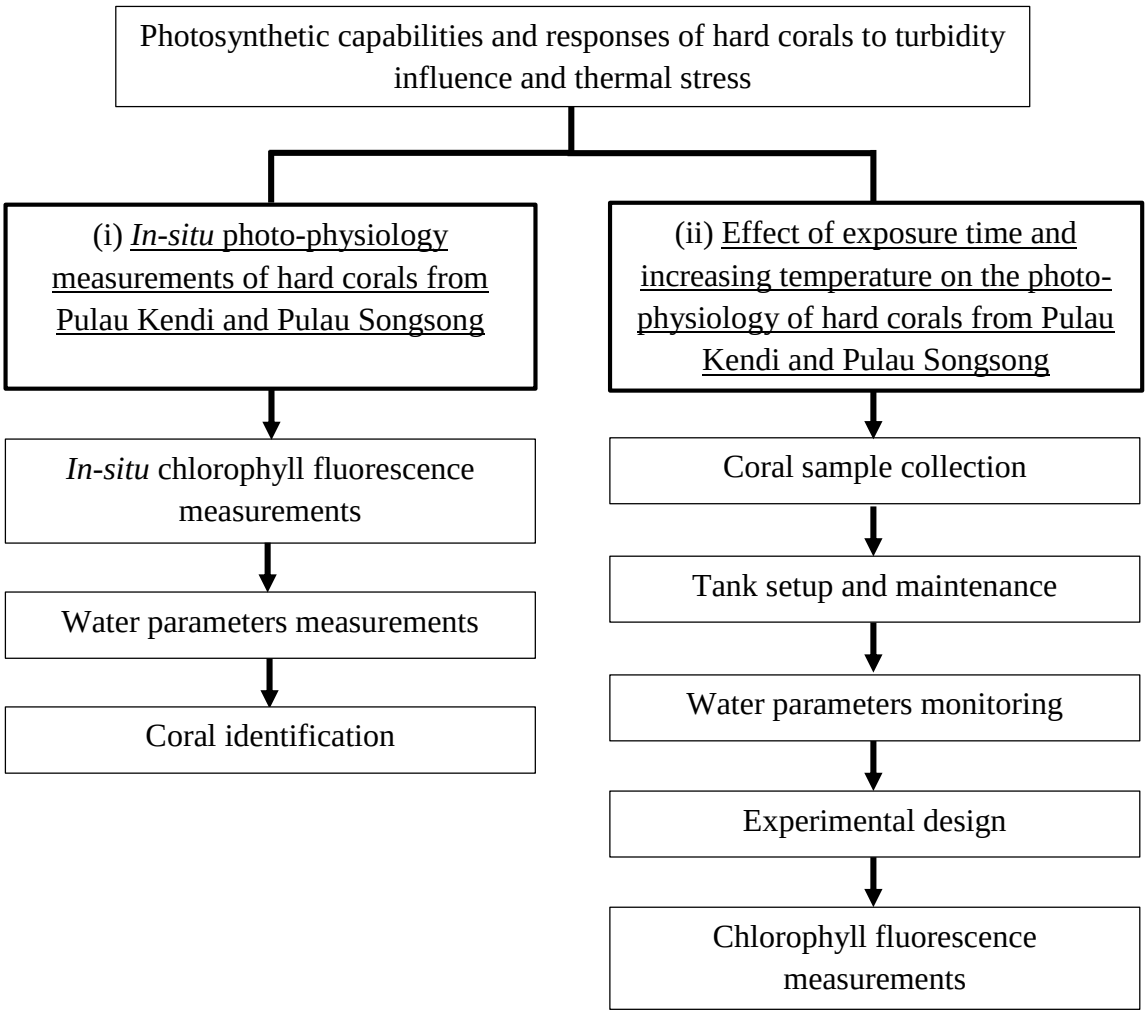

Figure 3.1 Flow chart on summarized methodology

3.1 Study site description

The study sites selected for *in-situ* photo-physiology measurements sampling were the coral reef areas of Pulau Kendi and Pulau Songsong (Figure 3.2) lies in the Northern Straits of Malacca. Pulau Kendi (5°13'58.44" N, 100°10'45.84" E) is a small island located at the southern coast of Pulau Pinang. Pulau Pinang is a state located at the west coast of Peninsular Malaysia. Pulau Kendi is a rocky island located 19 km Batu Maung coastal area which hard corals can be found at 4 to 5 metre depth of the island. Pulau Kendi is uninhabited but often visited by anglers. In addition, Pulau Kendi is exposed to nearby coastal reclamation at Batu Maung. Another study site is Pulau Songsong (5°48'42.12" N, 100°17'44.88" E) located in the state of Kedah which is also located at the west coast of Peninsular Malaysia. This island is located 8 km from Yan coastal area and is often visited by anglers and tourists. Hard corals at Pulau Songsong also can be found at 4 to 5 metre depth. Pulau Songsong is exposed to coastal development such as housing area. Pulau Kendi and Pulau Songsong are well known as with high water turbidity. They are located approximately 64 km from each other.

Two study sites are chosen in this study to investigate the photo-physiology of corals in turbid water area. Pulau Kendi and Pulau Songsong are the only islands in the Northern Peninsular Malaysia in which hard corals can be found although the water is high in turbidity. In addition, there was no previous study on photo-physiology was done on corals from both sites. Therefore, this study is seen as an opportunity to fill in the knowledge gap on the photo-physiology of hard corals in both islands. Water parameters (Table 3.1) of both study sites were measured and recorded during sampling at 12 pm. The weather during sampling was clear.

Table 3.1 Water parameters measured at Pulau Kendi and Pulau Songsong. Values were means ± SD (n = 3).

Site	Dissolved oxygen (mg/L)	pH	Salinity (ppt)	Temperature (°C)	Light intensity (µmol m^{-2} s^{-1})	Total suspended solids (mg/L)
Pulau Kendi	5.707 ± 0.091	7.853 ± 0.006	31.0 ± 0.0	29.4 ± 0.0	24.707 ± 1.329	95.653 ± 4.274
Pulau Songsong	6.233 ± 0.032	7.880 ± 0.010	29.0 ± 0.0	29.9 ± 0.0	54.267 ± 1.739	52.400 ± 11.504

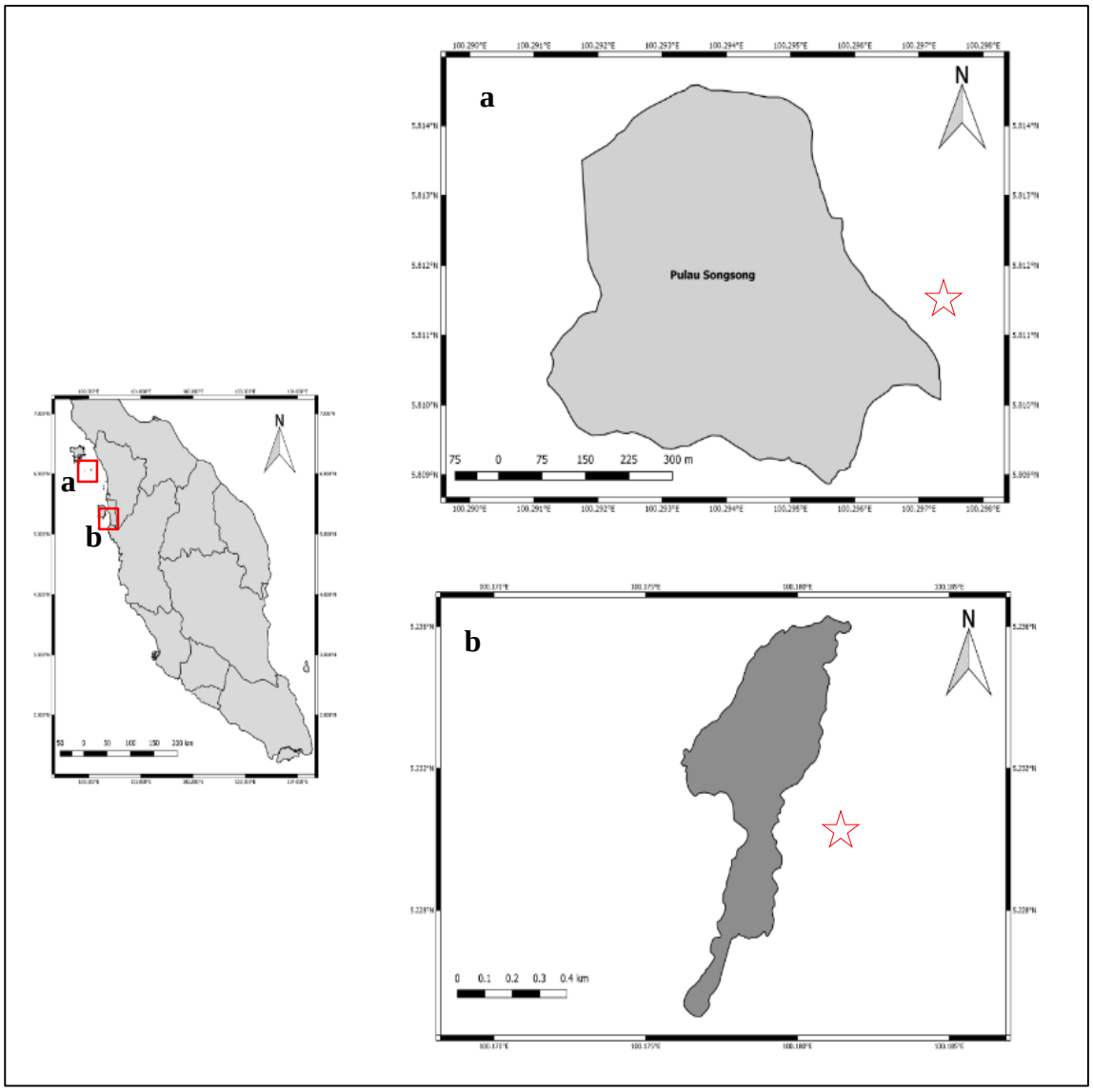

Figure 3.2 Map of Peninsular Malaysia showing Pulau Kendi 5°13’58.44” N, 100°10’45.84” E (a) and Pulau Songsong 5°48’42.12” N, 100°17’44.88” E (b) indicated by red square symbol. Location of sampling at each site were indicated by star symbol.

3.2 *In-situ* photo-physiology measurements of hard corals from Pulau Kendi and Pulau Songsong

3.2.1 *In-situ* chlorophyll fluorescence measurements

Photosynthetic parameters (F_v/F_m, $F_{v'}/F_{m'}$, rETRmax, α, E_k, qP and NPQ) of *Turbinaria mesentria*, *Goniastrea retiformis*, *Porites lutea* and *Goniopora cellulosa* from Pulau Kendi and *Goniastrea retiformis*, *Pavona danai*, *Porites lutea* and *Cyphastrea chalcidicum* were measured during sampling. All of the species are dominant species of both sites and they were chosen to determine the differences of photosynthetic parameters between species. The highest and least resistance species can be determined by measuring the photo-physiology of different species at each site. Species measured from both sites were different except for *Porites lutea*. All measurements of various species of hard corals were measured *in-situ* using an underwater Diving-PAM underwater fluorometer (Walz, Effeltrich Germany) while SCUBA diving. Before measurements, a modified version of leaf clip holder by Walz's was attached to the coral surface for dark-adaptation. The samples were dark adapted for 15 minutes to obtain maximum quantum yield. Dark adapted is condition which samples are kept in the dark to prevent from exposure to light to obtained accurate F_v/F_m reading. When samples were dark adapted, no electrons were transferred to PSII reaction centres due to no light energy was absorbed by light antennae. During this period, electron transfer stops, thus eliminating the trans-thylakoid pH gradient and allowing the full reduction of the primary electron acceptor Q_A. The maximum quantum yield of PSII is defined as:

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

The Diving-PAM fluorometer was set to deliver red pulse measuring light (0.15 µmol m^{-2} s^{-1}) to stimulate chlorophyll *a* fluorescence and to measure the minimum fluorescence yield (F_o, open PSII reaction centres) (Ralph *et al*., 1999). This was followed by a rapid light curve, a light treatment with an increasing series of eight consecutive actinic light steps. Each actinic light step was delivered for 10s (Ralph *et al*., 1999). The light emitting diodes provided the eight stepwise increments of actinic light at 0, 221, 320, 525, 793, 1318, 1852, 2000 and 3000 µmol m^{-2} s^{-1}. Other important Diving-PAM settings were: actinic light factor = 0.5, light curve intensity = 1, saturation width = 1, saturation intensity = 1, gain = 2 – 4 and signal damping = 3. Relative electron transport rate (rETR) at a given irradiance is given by:

$$rETR = \Delta F/F_{m'} \times PAR$$

Rapid light curves (RLCs) were generated from the calculated ETRs and the irradiances applied during the rapid light curve steps. Each RLCs was fitted to a double exponential decay function in order to quantify the characteristic parameters such as rETRmax, α and E_k (Platt *et al*., 1980; McMinn & Hegseth, 2004; McMinn *et al*., 2005; Ralph & Gademann, 2005). The initial slope of the RLCs (α) is a measure of the light harvesting efficiency of photosynthesis and the asymptote of the curve. The maximum rate of photosynthesis (rETRmax), is a measure of the capacity of the photosystems to utilize the absorbed light energy while E_k is the photoacclimation index (Marshall & Flynn, 2000; Ralph & Gademann, 2005) .

Non-photochemical quenching (NPQ) was also obtained during the measurement. NPQ is a measurement of amount of excess light energy that is dissipated as heat in the

PSII antennae system (Demmig-Adams & Adams, 1992). NPQ functions as photo-protective mechanism to protect against over-reduction of the electron transport chain. qP and NPQ was determined by the following equation:

$$qP = (F_m\text{-}F)/(F_{m'}\text{-}F_o)$$

$$NPQ = (F_m\text{-}F_{m'}) / F_{m'} \text{ (Schreiber, 2004)}$$

3.2.2 Water parameters measurements

Water temperature (°C), dissolved oxygen (mg/L) and pH from sea water of Pulau Kendi and Pulau Songsong were measured using a built-in oxygen and pH meter (YSI Pro-1020 USA) at 4.5 metre depth. Salinity (ppt) and underwater light intensity (µmol m^{-2} s^{-1}) were measured using a refractometer (Atago S/mill-E, USA) and a light meter with spherical underwater quantum sensors (LI-COR Biosciences USA) respectively. All water parameters were collected to determine the environmental condition during sampling which plays an important role in the photo-physiological process of corals.

3.2.3 Coral identification

Coral fragments of $5cm^2$ were collected from each species during sampling using chisel and hammer. In the laboratory, the fragments were soaked in a sodium hypochlorite solution with a 3:1 ratio of bleach and water to get a better view of corallites under the microscope. The colour of corals would slowly fade and completely turn white after three days. The fragments were then rinsed and dried at room temperature for another few days. A dissecting stereomicroscope (Olympus, SZ61TR-CCD, Japan) was used to capture the image and size of corallites for identification. Coral identification was determined using the key identification by Kelly (2011) and Veron, (2000).

3.3 Effect of different exposure and increasing temperature on the photo-physiology of different hard coral species from Pulau Kendi and Pulau Songsong

3.3.1 Coral samples collection

Four hard coral species, *T. mesenterina, G. minuta, P. lutea* and *G. cellulosa* were collected from Pulau Kendi and Pulau Songsong by SCUBA diving. The samples were collected in fragments with size of 5 cm^2 by using pruner, hammer, and chisel. The fragments were placed a in container filled with sea water and were transferred to CEMACS laboratory within two hours after sampling. Sampling methods are similar for both sampling sites. In the hatchery, the samples were kept in a large tank with a volume of 1100L supplied with flow through filtered sea water. The tank used was circular fiberglass with height of 1 meter and diameter of 1.5 meter.

3.3.2 Tank setup and maintenance

A culture tank supplied with filtered sea water and compressed air was prepared in the nursery to place hard coral samples which will be used for the temperature experiment. The flow of filtered sea water was continuous to regulate dissolved oxygen in the tank. Transparent roofs were used to ensure the corals receive enough light penetration. Average light intensity in the laboratory was 98.760 ± 2.352 μmol s^{-1} m^{-2}. To imitate light condition at natural environment, no light was supplied during the night. Corals were acclimatized in the tank for one week before experiments were started. One week is enough for corals to acclimate because the zooxanthellae are able to adapt to environmental condition within hours to days.