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The Effects of Temperature, Irradiance and Nutrient Stress on Maximum Quantum Yield of 3 Scleractinian Corals

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The Effects of Temperature, Irradiance and Nutrient Stress on Maximum Quantum Yield of 3 Scleractinian Corals

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Abstract

Coral bleaching is the major threat to coral reefs worldwide. Coral reefs, as an essential part of the marine ecosystem, are under severe threat from many sources, including high sea water temperatures, solar irradiance and anthropogenic impacts such as nutrient enrichment, sewage disturbance and others. Malaysian corals were affected during the global mass bleaching events in 1998 and 2010 which caused bleaching and some mortality, but coral reef degradation is also due to other causes. Mostly Peninsular Malaysia coral reefs are affected by agricultural activities and human development causing sedimentation and nutrient runoff. To date, the photosynthetic performance of Malaysian corals under such stresses is unknown, and laboratory experiments were conducted to assess how some commonly occurring corals of that region respond to stress factors. The objectives of this study were: (1) To record maximum quantum yield of chlorophyll fluorescence (F_v/F_m) of coral species; *Stylophora pistillata, Montipora digitata and Seriatopora hystrix* in treatments of stressors of high/ambient temperature-light levels and high/ambient temperature-nitrate levels, (2) To record maximum quantum yield of chlorophyll fluorescence (F_v/F_m) of coral species in stress treatments of combinations of high/ambient temperature-light levels and high/ambient temperature-nitrate levels, (3) To differentiate changes in quantum yield fluorescence among the three corals species before stress, after stress and after 24-h recovery stage. Colours and paling were also being examined by using CoralWatch Coral Health Chart. To achieve this, *S. pistillata, M. digitata and S. hystrix* were exposed to: a) ambient (27°C, 200 µmol quanta $m^{-2}s^{-1}$), (b) high light (27ºC, 520 µmol quanta $m^{-2}s^{-1}$), (c) high temperature (30°C, 200 µmol quanta $m^{-2}s^{-1}$) and (d) high temperature + high light (30°C, 520 µmol quanta $m^{-2}s^{-1}$). In a further investigation, the overnutrification factor was introduced: (a) ambient control (27ºC, 2 µM NO_3^-), (b) high

nitrate (27ºC, 15 µM NO_3^-), (c) high temperature (30ºC, 2 µM NO_3^-), (d) high temperature + high nitrate (30ºC, 15 µM NO_3). 20 minutes of dark-adapted photochemical efficiency (F_v/F_m) was measured using a pulse-amplitude-modulation (PAM) chlorophyll fluorometer (WATER-PAM, Walz, Germany). Besides, here it includes the study of Reef Check data and relates it with 50-km monitoring product of Degree Heating Week Coral Reef Watch for 2009 until 2011 in Peninsular Malaysia and East Malaysia to predict bleaching behaviours within Malaysian waters. For the temperature-light stress treatments, there were significant decreases in maximum quantum yield for all species, due to photoinhibition. The results show that *S. hystrix* is susceptible to thermal stress. In temperature-nitrate stress experiments, it is suggested that nutrient enrichment may not have a synergistic effect, and that high temperatures alone significantly impact F_v/F_m values (three-way ANOVA, $p>0.05$) for all coral species. Slow growing corals (*S. pistillata*) appear to cope better with the environmental changes than the fast-growing corals (*M. digitata* and *S. hystrix*). This research helps understand the effects of coral bleaching and nitrate stress and may be of value to researchers and managers of marine parks in the Malaysian region to better understand coral health.

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Chapter 1

Introduction

1.1 Coral-zooxanthellae relationship

Animal-algal endosymbioses are the combination of heterotrophic (animal host) and autotrophic (algal symbiont) organisms into single functional units (holobionts) that contribute substantially to coral reef productivity (Rowan, 1998). Hermatypic corals flourish in tropical waters that are considered nutrient poor, a fact usually attributed to their symbiosis with zooxanthellae (Jackson et al., 1989). Hermatypic corals are mainly responsible for the modern reef formation that succeeds in oligotrophic waters (Hume et al., 2013). Zooxanthellae is a general descriptive term for all symbiotic golden-coloured algae that live in animals, including corals, sea anemones, molluscs and other taxa. Scleractinian reef building corals are also referred to as zooxanthellate corals. Gymnodinoid dinoflagellates in the genus *Symbiodinium,* known as zooxanthellae, is the dominant algae in symbiosis with marine benthic Cnidaria (Goodson *et al*., 2001).

The host Cnidarians have a very simple two-tissue-layer body plan where they harbor the symbionts intracellularly in vacuoles (symbiosomes) within cells in the inner or gastrodermal tissue layer (Weis, 2008). Zooxanthellae exist in new corals from both asexual and sexual production (Birkeland, 1996). For sexually-produced corals, the zooxanthellae are either directly inherited from the parent or indirectly from the environment (Birkeland, 1996). Zooxanthellae swallowed into the gastrovascular cavity of a new coral polyp are transported into the gastrodermal tissues, where they begin to reproduce (Borneman, 2009). In asexually-

produced corals, the symbionts are directly transmitted in the coral fragments when forming a new colony (Birkeland, 1996). A coral which produces zooxanthellae-free larvae, will acquire new algal symbionts from its environment (Mieog et al., 2009). A few types of *Symbiodinium* are taken up by these corals, and only one zooxanthellae type will become the dominant, while others remain in low abundances (Mieog et al., 2009). *Symbiodinium* cells are golden brown due to the presence of light harvesting and photosynthetic pigments in their chloroplast (Weis, 2008). The zooxanthellae reside in specialized vacuoles within tissue cells in the gastrodermis (Borneman, 2009). While this is the inner layer of cnidarian tissue, the zooxanthellae are nonetheless still well within reach of light energy (Borneman, 2009). Healthy corals harbor millions of *Symbiodinium* per square centimeter of tissue and therefore have this same golden-brown hue (Weis, 2008). Zooxanthellae occur at densities of between 0.5 and 5 million cm^{-2} coral and translocate 95% of their photosynthate to their coral host (Smith et al., 2004). The symbiotic relationship consists of a cnidarian host providing a protective environment for the zooxanthellae in its tissues. Coral-zooxanthellae symbioses are mutualistic because both partners receive benefit from the relationship through nutrient exchanges (Stat et al., 2008). The nutrient exchanges mean inorganic waste metabolites produced by the animal host (i.e corals) are exchanged for organic nutrients fixed by dinoflagellates photosynthesis (Stat et al., 2008). *Symbiodinium* produce photosynthetic carbon and the fixed carbon is transferred to the animal host's tissues. They also recycle the animal's nitrogenous waste (Goodson et al., 2001). The algae recycle the nitrogen by assimilating ammonia into essential amino acid, a valuable compound to the host (Venn et al., 2007). The cnidarian host provides the zooxanthellae with a protective environment within its tissues, provide carbon dioxide, ammonium, urea and polyphosphates (Gustafsson et al., 2013).

Hermatypic corals flourish in tropical waters that are considered nutrient poor, due to their symbiosis with zooxanthellae (Jackson et al., 1989). Zooxanthellae with efficient photosynthetic performance are essential for the maintenance and growth of corals (Smith et al., 2004). Many corals (stony corals and octocorals) depend on their symbiotic algae for survival (Goulet 2006). Zooxanthellae have the capability of extracting nutrients such as nitrate, ammonium and phosphate from the water (Zubinsky and Stambler, 1996). Scleractinian coral symbiosis is also closely tied to the ability of the corals to deposit their calcium carbonate skeletons that form the reef structure (Weis, 2008). The high productivity and diversity of coral reefs is largely due to the mutualistic symbiosis between corals and zooxanthellae (Silverstein et al., 2012). The zooxanthellae contain chlorophyll which depends on the sunlight energy to build the amyloid assimilation product (Yonge, 1931). This brown unicellular algae endosymbiosis is a dominant feature of coral reefs and they are proven to help the existence of the coral reef ecosystem (Yonge and Nicholls, 1931; Rowan, 1998).

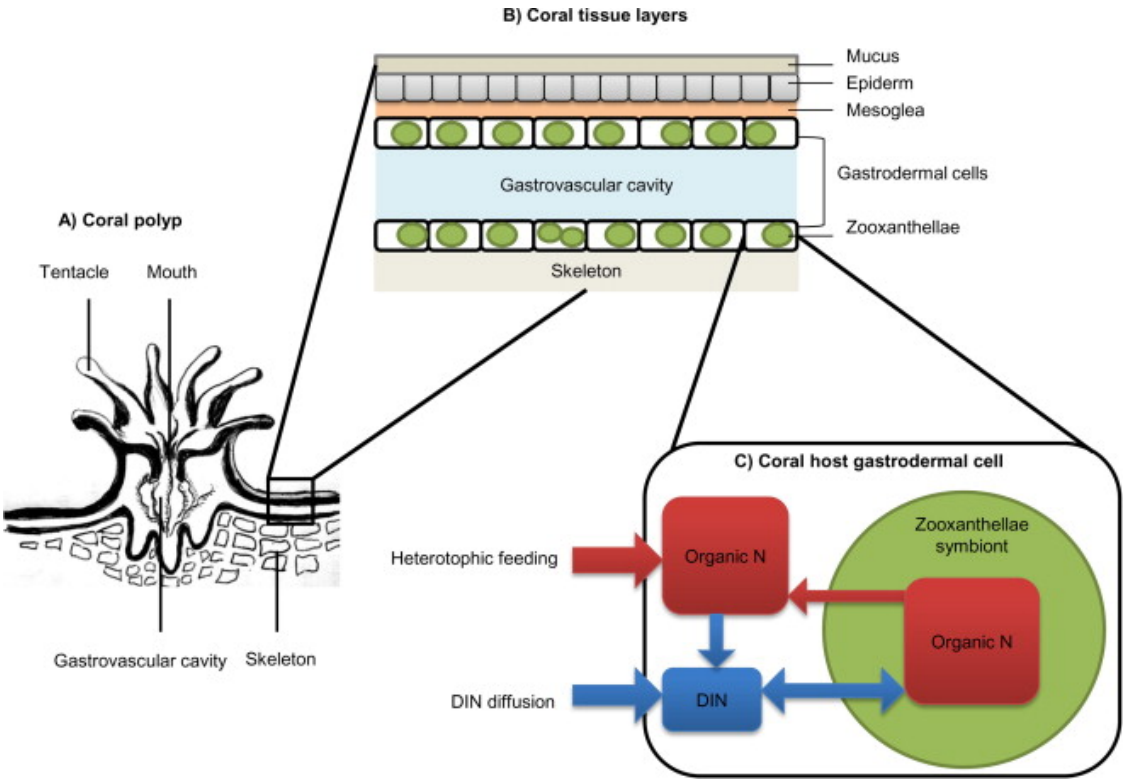


Figure 1.1: Schematic of coral tissue organisation. (A) General drawing of a coral polyp. (B) Coral tissue layers and organisation of zooxanthellae within the gastrodermal cells. (C) Flux of organic and inorganic nitrogen between the coral host and the zooxanthellae symbiont. (Gustafsson et al., 2013)

The symbionts of zooxanthellae are diverse, and include the members of classes Bacillariophyceae, Cryptophyceae, Dinophyceae and Rhodophyceae, and the most studied ones are dinoflagellates (Rowan, 1998). *Symbiodinium* occur in a variety of hosts and most of the cnidarians, found commonly in shallow subtropical waters (Baker, 2003). Among the cnidarian species that contain *Symbiodinium* are Anthozoa (i.e. anemones, scleractinian corals, zoanthids, corallimorphs, blue coral, alcyonacean corals and sea fans) and some Scyphozoa and Hydrozoa (Baker, 2003).

1.2 Coral holobiont and the role of different microorganisms in dictating coral health and success.

The coral holobion is a group of organisms which associated with and within the host coral animal (Rohwer et al., 2002). It is known to be distributed in the mucus, tissue and skeleton parts of corals (Massé, 2018). According to Radecker et al. (2015), the coral holobiant includes protozoans, fungi, archaea and bacteria. The mechanisms and interactions of coral and holobionts can modify the corals response towards environmental issues such as ocean acidification, ocean warming and euthrophication (Radecker et al. 2015). Radecker et al. (2015) mentioned that the coral holobiont acts as the nitrogen assimilator in the coral's symbiotic relationship. The coral holobiont can adapt to changing environmental conditions (Rosenberg et al., 2007). Rosenberg et al. (2007) thought that corals can be resistent to pathogens from the holobiont.

1.3 Photosynthesis in symbiotic animals

Photosynthesis is a process of converting solar energy to chemical energy and is important to our planet (Roth, 2014). Animals which have symbiotic algal cells contribute carbon dioxide and water for the photosynthetic reaction and all substrates required for the photosynthetic apparatus (Venn et al., 2007). Porifera (sponges) and Cnidaria (the hydroids, corals, sea anemones and jellyfish) may harbor photosynthetic algae which fix carbon dioxide and release oxygen (Venn et al., 2007). Symbiosis with zooxanthellae provide the host with photosynthetic products, allowing the coral to bloom and grow in oligotrophic tropical seas (Yamashita et al., 2014). Both zooxanthellae and the coral host are able to assimilate, reduce and involve into their metabolism inorganic compounds of nitrogen and phosphorus from sea water (Venn et

al., 2008). The zooxanthellae take up CO_2 and bicarbonate from the sea water for their photosynthesis process, and build the calcium-carbonated coral's skeleton (Venn et al., 2008). The coral host provide the symbiont with essential nutrients in a sunlit environment (Roth 2014). Zooxanthellae clades can differ in their functional responses to light, CO_2 and temperature (Suggett et al., 2017).

1.4 Corals clade and temperature tolerance

Reef building corals contain autotrophic endosymbiotic algae (zooxanthellae) in their gastrodermal cells (Muscatine and Cernichiari, 1969). Corals have a large surface area to volume relationship to maximize captured light by multiple layers of zooxanthellae, (Venn et al., 2008). From molecular DNA techniques, *Symbiodinium* sp. are categorized into groups of A, B, C and D (Dong et al., 2009). Biogeographically, *Symbiodinium* clades A, B and C are common in the Caribbean oceans, and clades C and D are common to most corals in Indo-Pacific waters (Dong et al., 2009). *Symbiodinium* are genetically diverse, consisting of eight major divergent lineages (clades A-H) (Wooldridge, 2010), while Baker (2003) reported only clades of A-F. Each clade contains multiple subclades, strains or types (Jones *et al*., 2008). The types of symbiont which are found in coral hosts is one of the factors that influence corals' thermal stress tolerance (Fitt et al., 2005). There are eight major clades that have been recorded among the symbiont *Symbiodinium* sp. (Fitt et al., 2005 and Coffroth and Santos, 2005) (*Symbiodinium* clades A, B, C, D, E, F, G and H), of which only four are related to reef-building corals (Fitt et al., 2005). The clade types can be sympatrically functioning within individual corals, for example; the genus *Montastraea* corals in Caribbean contain clades A and B dominated the exposed tops of coral colonies and clade C dominated the shaded sides of the colony (Rowan et al., 1997). While a study by LaJeunesse et al. (2003) found that *Stylophora*

pistillata at 3m water depth contain *Symbiodinium* C1. Brown et al. (2000) found that *Goniastera aspera*, an Indo-Pacific coral found on east and west surfaces had different of temperature and solar radiation tolerance because of algal genotypic differences. These distributions of *Symbiodinium* pairings are usually correlating with temperature and solar radiation (Coffroth and Santhos, 2005).

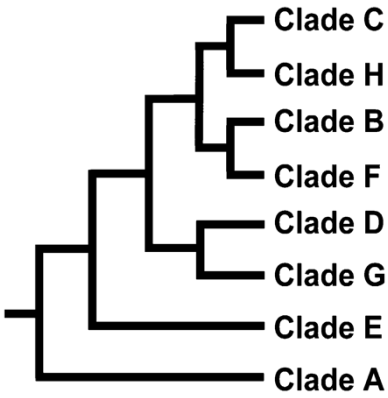


Figure 1.2: The phylogenetic relationships between the major clades of *Symbiodinium*. All clades except clades E and H are within scleractinian corals, which clades A, B, C, and are the predominant symbionts with scleractinian corals (Coffroth and Santhos, 2005).

Coral reefs depend on symbiotic algae that function to restrict the outward flow of life-supporting nutrients to the water column; corals and the zooxanthellae live by limiting the flow of nitrogen and other essential nutrients to the nutrient 'desert' represented by tropical seas (Hoegh-Guldberg, 1999). Corals reefs obtain the nitrogen for their growth, tissue repair, mucus production, and reproduction (Badgley *et al*., 2006).

Certain zooxanthellae clades group differ in light harvesting and utilisation, their ecological diversification (Suggett et al., 2017). Referring to Suggett et al. (2017), clades A to I are different based on the divergence of small ribosomal subunit RNA, internal transcribed spacer regions (ITS), choloroplast large subunit (cp23S) and cytochrome oxidase b (cob). For

example, ITS2 type C3 is harboured by Acropora species which is known as highly stress sensitive (Sugget et al., 2017).

1.5 Coral bleaching

Coral bleaching can be defined through photoacclimation, where the response of zooxanthellae numbers and the host coral colour (Suggett and Smith, 2011). Coral bleaching can also be related with thermal stress; elevated ROS production by host tissues (Suggett and Smith, 2011). Coral bleaching is defined sublethal stess which does not bring mortality and lethal bleaching which resulted in coral mortalty (Suggett and Smith, 2011). However, it is common to see a thermal anomaly, bleaching and mass coral mortality (Suggett and Smith, 2011).

The coral-zooxanthellae symbiosis is very sensitive to increases in temperature, and changes as little as 1°C above the average summer maximum for sustained periods can lead to a breakdown of the symbiosis (Mieog et al., 2009). The endosymbiotic dinoflagellate microalgae are very sensitive to elevated water temperature and the thermal sensitivity of the symbiotic algae of corals is an important physiological feature, because it is believed to be the underlying cause of the phenomenon of coral bleaching (Jones et al., 2000). It has been accepted that the impairment of the photosynthetic machinery of the zooxanthellae is the proximal driver of the thermal bleaching response (Wooldridge, 2013), but the initial site of damage and early dynamics of the impairment are not well studied.

Bleaching is a condition where the coral loses their algal symbionts and pigments, in response to environmental stresses (Fitt et al., 2001) and the stress levels depend on the temperature, exposure length and other environmental factors, such as light, salinity and also water motion.

Zooxanthellae loss effects corals because the symbionts supply 63% of the coral's nutrients (Banin et al., 2000).

High sea surface temperatures have been suggested as the primary cause of mass bleaching events (Hoegh-Guldberg and Smith, 1989). Jones (2008) mentioned natural bleaching events are usually caused by seasonal cycles in algal density, long warm summers which can stress the corals and increase coral mortality. Irradiance is also considered as one of the main environmental triggers of bleaching (Fitt et al., 2001). Light has been known as the important key factor for the productivity, physiology and ecology of the coral-algal symbiosis (Roth, 2014). The symbiosis requires the right light quantity (photon flux) and quality (spectral composition), combined with depth (mostly < 30 m) and skeletal structure, to ensure the success of the photosynthesis process (Roth, 2014). Corals tolerate less than 2000 μmol photons m^{-2} s^{-1} at midday (Jimenez et al., 2012)

In an environment disturbed by especially elevated temperature or irradiance, the *Symbiodinium* may produce the reactive oxygen species (ROS) which may damage the membranes and proteins of corals and later lead to 'bleaching' (Venn et al., 2008). When 'bleaching' occurs, there has been a breakdown of the symbioses, with the loss of algae pigments or algal cells from the coral host. Bleaching conditions may lead the corals towards growth reduction, slow reproduction, diseases, or in the worst condition, death (Venn et al., 2008).

If coral bleaching incidences continue, they will result in a decrease in growth and reproduction, and an increase in mortality rates for corals repeatedly bleached (Lesser, 1996). Prolonged bleaching can lead to coral mortality and can devastate entire reefscapes

(Wooldridge and Done, 2009). In the laboratory, bleaching can be triggered by multiple factors: extremes of temperature (heat and cold shock), high irradiance, prolonged darkness, heavy metals and pathogenic micro-organisms (Douglas, 2003). When the bleaching is severe, the symbiosis is unable to re-establish itself and the coral may die (Mieog et al., 2009). The condition of temperatures higher than normal for an environment can affect the photosynthetic mechanism of the symbiotic algae (Mostafavi et al., 2007). Environmental variables, such as elevated sea temperature, solar radiation and reef depth give different susceptibilities for individual coral species (Yee et al., 2008). Some coral species are more resistant than others to environmental factors that cause bleaching and bleaching-related mortality (Stimson *et al.,* 2000). Differences in sensitivity of species to disturbance can lead to community shifts and loss of coral reef diversity (Yee et al., 2008). Different species and genera which survived (or not) the bleaching event are related to the characteristics of the host-symbiont associations in those taxa. The host-symbiont association has been described as the rate of release of degraded zooxanthellae, the density of zooxanthellae per square centimeter of coral surface area, and the mitotic index of zooxanthellae (Stimson et al., 2002). Symbiont types were found to differ in their photosynthetic response to light and temperatures, such as *Symbiodinium* C- a symbiont type in *Acropora* coral on the Great Barrier reef, sensitive to heat stress (Jones et al., 2008), and *Symbiodinium* D which is heat stress tolerant (Jones et al., 2008). The characteristics of the host-symbiont association also can separate corals based on their bleaching susceptibility, such as *Acropora* spp. and *Seriatopora hystrix* as high mortality, *Montipora* as low mortality and *Porites* spp. a massive coral is characterized as the lowest mortality (Stimson et al*.,* 2002).

1.6 Coral Photoinhibition

Photoinhibition has been studied for over a century using biochemical, biophysical and genetic methodologies (Adir et al., 2003), and the current understanding of photoinhibition is damage primarily associated with photosystem II (PSII), particularly with the increased loss of the D1 protein of the PSII reaction centre (Hoegh-Guldberg and Jones, 1999). Kok (1959) reported that the damage 'sustained' to photosynthetic systems was measured originally as a long-lasting decrease in photosynthetic rate (oxygen flux) at high irradiances. Osmond (1994) reported that photoinhibition is measured as a reduction in photosynthetic efficiency (quantum yield), also known as maximum rate of photosynthesis, under high-irradiance conditions. A decade ago, there were two opinions on photoinhibition; (1) Focusing on photoinactivation when excess excitation reached the PSII reaction center (Osmond and Förster, 2008); and (2) focusing on regulatory processes in the antennae that reduced excitation transfer to PSII, thereby achieving photoprotection (Osmond and Förster, 2008).

Photoinhibition is a physiological state of stress that occurs in all oxygen evolving photosynthetic organisms exposed to too much light (Adir et al*.,* 2003) causing a decrease in the capacity of the photosystem to capture and process photons (Hoegh-Guldberg and Jones, 1999). Photoinhibition studies are synonymous with light-related decreases in measurements of quantum yields (F_v/F_m) after periods of dark adaptation (Hoegh-Guldberg and Jones, 1999; Bhagooli and Hidaka, 2004). In high light intensities, increasing light levels causing light reactions to over-reduced and potentially harmful products such as oxygen free radicals are produced (Hoegh-Guldberg, 1999). The oxygen free radicals will eventually lead to cellular damage if it is not detoxified by several enzyme systems (Hoegh-Guldberg, 1999).

Photodamage is recognisable as: (1) photosynthetically active light produces reactive oxygen species (ROS) either by excessive reduction of Q_A (the primary electron acceptor of PSII), (2) by charge recombination between the acceptor side and the donor side of PSII (Murata et al., 2007). When Q_A becomes highly reduced (during light intensities above the photosynthetic system needs), the rate of damage can exceed the rate of repair. PSII will lose its function and the photosynthesis will decline (Smith et al., 2005). Photodamage not only occurs during high light intensities, but also when light intensities are below those normally required to saturate photosynthesis in conjunction with other factors such as elevated temperature, which limit the ability of zooxanthellae to acquire and assimilate CO_2 (Smith et al., 2005).

The two independent mechanisms of PSII photoinactivations, acceptor and the donor side can cause degradation and programmed turnover of the D1 protein. Donor side photoinhibition is not occurring in cells related to coral bleaching (Smith et al., 2005). For acceptor side inhibition, when there are high light intensities, the plastoquinone pool is reduced (Smith *et al*., 2005). The process will continue and involves the reaction center of chlorophyll, and singlet oxygen, which later react with the D1 protein (Smith *et al*., 2005). The singlet oxygen might damage PSII (Tyystjärvi, 2008) because it will react with D1 protein and triggering its degradation (Smith et al., 2005). Under excess light conditions, D1 content has been found to decrease in coral zooxanthellae (Smith et al., 2005).

Based on their recovery times, photoinhibition can be distinguished into two types; dynamic and chronic photoinhibition (Garbunov et al., 2001, Bhagooli and Hidaka, 2004). Chronic photoinhibition is associated with much more severe damage to PSII, recovers extremely slowly, and is interpreted as photon damage (Garbunov et al., 2001). Chronic photoinhibition has been observed under unfavourable environmental conditions, such as low or high

temperature, limited CO_2 supply, or nutrient limitation (Garbunov et al., 2001). A few studies suggest that a constant daytime exposure to high doses of visible and UV radiation can lead to chronic photoinhibition, even at moderate temperatures (Garbunov et al., 2001; Winters et al., 2003).

Dynamic photoinhibition is rapidly reversible within hours while chronic photoinhibition might be reversible within days or irreversible (Garbunov et al., 2001). Dynamic photoinhibition describes all reactions that decrease the efficiency of photosynthesis when zooxanthellae are exposed to light. It is attributed to transient damage to the reaction centers of PSII, recovers relatively rapidly and can serve as a photon protection mechanism (Garbunov et al., 2001). Dynamic photoinhibition occurs over a diurnal irradiance cycle (Hoegh-Guldberg and Jones, 1999). When there is decline in the photochemical efficiency during and/or after exposure to high irradiance, in dynamic photoinhibition it is due to reversible processes such as increase dissipation of absorbed light as heat (Winters et al., 2003). This type of photoinhibition is also known as non-photochemical quenching, and it is an inhibition which followed by photoprotection (Lesser, 2004). In chronic photoinhibition, when there is damage to the PSII, D1 protein will degrade, but for dynamic photoinhibition, the excess absorbed light energy will be controlled by carotenoids (Hanelt, 1998).

The underlying mechanisms involved in photoinhibition, which causes coral bleaching will be a number of different mechanisms, depending on coral species (zooxanthellae ecotypes and clades), physiological state and environmental conditions (Smith et al., 2005). Other than thermal stress, host-symbiont associated organisms are also exposed to unfavorable environmental conditions such as high salinity, low temperatures (Murata et al., 2007), high

nutrients and sedimentation (Faxneld et al., 2010). Future studies should therefore be conducted on how various stresses can affect photodamage and repair (Murata et al., 2008).

1.7 Corals and Nitrogen

Nitrogenous nutrients, in surrounding coral reef waters, are usually extremely low in concentration (Webb, 1975). But, elevation of inorganic N may influence the symbiont algae's density, division rate and also their release rates (Stimson and Kinzie, 1991) and any disturbance of microbial nitrogen cycling may be linked to coral bleaching and disease (Rädecker et al., 2015). Algal symbionts are highly dependent on nitrogen availability to sustain primary productivity (i.e., photosynthesis) (Rädecker et al., 2015) because they uptake dissolved inorganic nutrients (DIN) from surrounding seawater in the form of ammonium (NH_4^+) and nitrate (NO_3^-). These nutrients are partially translocated to the host as amino acids (organic nitrogen compounds) and the rest is stored or used in their metabolism activity (Rädecker et al., 2015).

Besides global climate change, anthropogenic causes such as eutrophication, coastal development, sedimentation and over-fishing are responsible for the decline in the health of coral reefs (Wilkinson, 1999). Eutrophication on coral reefs was highlighted in the 1960s and 1970s, related to a case study in Kaneohe Bay, Hawaii where waters bloomed with phytoplankton caused by sewage discharge (Szmant, 2002). In Australia, corals on the Great Barrier Reef damaged by anthropogenic influences, included eutrophication, the situation where nutrient levels have occurred through human activities (i.e. sewage discharge) (Bell, 1992). A study by Wear and Thurber (2015) recorded that sewage is one of the major causes of coral reef degradation. Eutrophication may cause degradation of coral reefs and in time, the coral community will be replaced by attached algae, seagrasses or filter feeders (Bell, 1992). The water quality indicator for eutrophication that has been widely used is the concentration of chlorophyll a (Bell, 1992). Eutrophication can lead to increased phytoplankton

concentration, reduced light penetration to the symbiotic algae, and death due to smothering phytoplankton (Bell, 1992). Eutrophication also can cause a rapid development of attached algae which will compete with corals for space (Bell, 1992). Eutrophication was also found to be the cause of reduction of coral growth rates and their larval settlement, in the inner to mid Great Barrier Reefs lagoon (Bell, 1992). In Sanya Bay, China, rapid development of aquaculture has caused eutrophication in the ocean, bringing about an increase in inorganic nitrogen and phosphorus (Baohua *et al*., 2004). In the Caribbean region, it is found that eutrophication is a significant contributor to the decline of coral calcification (Gardner et al. 2003), increasing coral disease rates (Bruno et al. 2003), coral-algal competition decreasing coral reproduction and recruitment (Fabricius, 2005; Fabricius, 2011; Pastore, 2014). Eutrophication and nitrification has been a problem in the Indo-Pacific region especially in the vicinity of large human settlements (Szmant, 2002), with high levels of waste-water discharges (sewage, run-off, groundwater seepage (Bell, 1992). Elevated nutrients can affect coral reef health through direct physiological effects on the corals, such as increased susceptibility to bleaching or disease mortality when there are increases in algal turf biomass and productivity which declined the coral's health (Szmant, 2002, Duprey et al., 2016). According to Baohua et al. (2004), excessive inorganic nitrogen can result in coral bleaching, where 0.001 mmol/L concentration of ammonia or nitrate had significantly increased the symbiotic algae expulsion. Faxneld *et al*. (2010) pointed out that corals exposed to multiple stressors (high temperature in combination with low salinity and high nitrate) had caused mortality within 24h of exposure. Laboratory or field experimental work on exposing corals to natural or anthrophogenically-elevated nutrient concentrations can be undertaken to study the effects of nutrients on the physiological corals (Szmant, 2002).

Table 1.1: Laboratory studies of direct effect of nutrients on coral physiology in aquaria for periods of a few weeks to months. The concentrations used were the magnitude higher that the highest levels of measured on polluted coral reefs (Tomascik and Sander, 1987).

Studies by	Nutrient enrichment	Corals	Duration	Result
Atkinson et al. 1995	5 µM nitrate, 2 µM ammonium and 0.6 µM phosphate	Hermatypic corals		Higher long-term corals growth rates
Snivdongs and Kinzie 1994	+15 µM ammonium	*Porites cylindrica*	8 weeks	Increased cellular chlorophyll *a*
McGuire and Szmant 1997	10 µM ammonium	*Porites asteroides*	2-4 weeks	Decreased in zooxnthellae C:N ratios and calcification
Marubini and Davies 1996	1, 5 and 20 µM nitrate	*Porites porites* and *Montastrea annularis*	4 weeks	25-50% decrease in growth rate
Marubini and Atkinson 1999	5 µM nitrate	*Porites compressa*		No effect on growth Reductions in calcification (with decreased pH of seawater)
Ferrier-Pages et al. 2000	20µM ammonium 2 µM phosphate	*Stylophora pistillata*	10 weeks	Slow recovery to normal condition

The symbiotic relationship with nutrient recycling and a close relationship between trophic levels have been the key to the ecological success of reef building corals (Smith *et al*., 2004). Therefore, any factor that reduces the efficiency of this symbiotic relationship will have a major effect on Scleractinian corals, consequently the coral reefs productivity (Smith *et al*., 2004).

1.7.1 Corals and Nutrient Enrichment

Coral reef degradation resulting from nutrient enrichment of coastal waters is of increasing global concern. The effects of nutrients on coral reef organisms have been demonstrated in the laboratory, rather than their actual effect on coral reef biota *in situ* (Koop et al., 2001). Increasing urbanization of coastal areas, which are often associated with loss of forest and coastal wetlands and increased intensive agricultural activities have led to an increase in the rate of land runoff, loaded with sediment and nutrients from fertilizers which are then discharged into coastal waters after heavy rains (Koop et al., 2001). Szmant (2002) reported that NOAA had identified sources of 'runoff stressors', such as land cover conversion, land clearing, fertilizer application, sewage leakage, effluent discharge, pesticide application, industrial pollution, seafloor dredging and aquaculture discharge. Anthropogenic nutrient enrichment is more likely to affect coral reefs closer to shore, within lagoons or embayment (limited circulation and flushing), and reefs associated with larger land masses, especially near significant human populations.

Coral reefs typically flourish in water that is oligotrophic (nutrient-poor). Coral reefs prone to nutrient effects are usually exposed to other anthropogenic stressors than can make a reef more susceptible to nutrient effects or cause symptoms similar to those expected from nitrification (Szmant, 2002). Changes in nutrient concentrations in coral reef areas can fundamentally alter

the food web in the ecosystems where a high concentration of nutrients promote the proliferation of plankton, then can lower light transmission and slow the activity of symbiotic algae living in coral tissues, thus slowing coral growth rates (Larsen and Webb, 2009). Szmant (2002) stated that the elevated nutrients may also affect physiological interactions between corals and their zooxanthellae by stimulating the cell division rates of the intracellular symbionts. This might be similar to the findings of Muscatine et al. (1989), suggesting that the availability of inorganic nitrogen (20 µm ammonium and 2 µm phosphate) can lead to increased protein synthesis in zooxanthellae, and an increase in zooxanthellae numbers. Nordermar et al. (2003) concluded that corals on nutrient-exposed reefs to +15µM for 14 days may be more susceptible to periods of elevated temperature (34ºC), illustrated by a decrease in primary production rate of zooxanthellae. Marubini and Davies (1996) found that elevated nitrate concentrations (1, 5 and 20µM) are responsible for the decline in calcification, in *Porites porites* and *Montipora annularis*, and by extrapolation, in the rate of growth of reefs as a whole. A laboratory study showed a long-term effect (12 months) of inorganic nitrogen enrichment (40 µM of NH_4^+ and 30 µM of NO_3^-) on *Stylophora pistillata* and *Acropora* spp., affecting growth rates, chlorophyll *a* content and the zooxanthellae density (Yuen et al., 2008). Their studies suggested that nutrient enrichment is not an only cause for coral reef degradation but could result in synergistic impacts when corals are exposed to other environmental stressors.

1.8 Malaysian reefs

Wilkinson et al. (1993) reported that about 60% of corals in Southeast Asia are on the edge of destruction and predicted that most of the reefs in the region will be eradicated within the next 40 years. In the last 20 years, high levels of development and land-use changes have been the

major threats to coral reefs in the region of South East Asia (Burke et al., 2002), including deforestation, constructions of roads, airports, building (tourist resorts are the main cause), increasing sediment and nutrient loads in the coastal areas (Burke et al., 2002). Coral reefs in marine parks (which are not always strict marine reserves) throughout Malaysia have also been exposed to human impacts such as unsustainable fishing activities and effects of dive and snorkelling sites (Mazlan et al., 2005).

Malaysia is composed of Peninsular Malaysia (Figure 1.3), Sabah and Sarawak (Figure 1.4), located in the Coral Triangle of the Indo-Pacific region (Mazlan et al., 2005). Malaysia has one of the largest continental shelf areas within the tropical world (Mazlan et al., 2005) and the reefs in this area have a rich assemblage of marine life, including 76% of the world's coral species. Data on corals in Malaysia was recorded as early as 1970 but was not reported and dispersed among institutions (Tun et al., 2004).

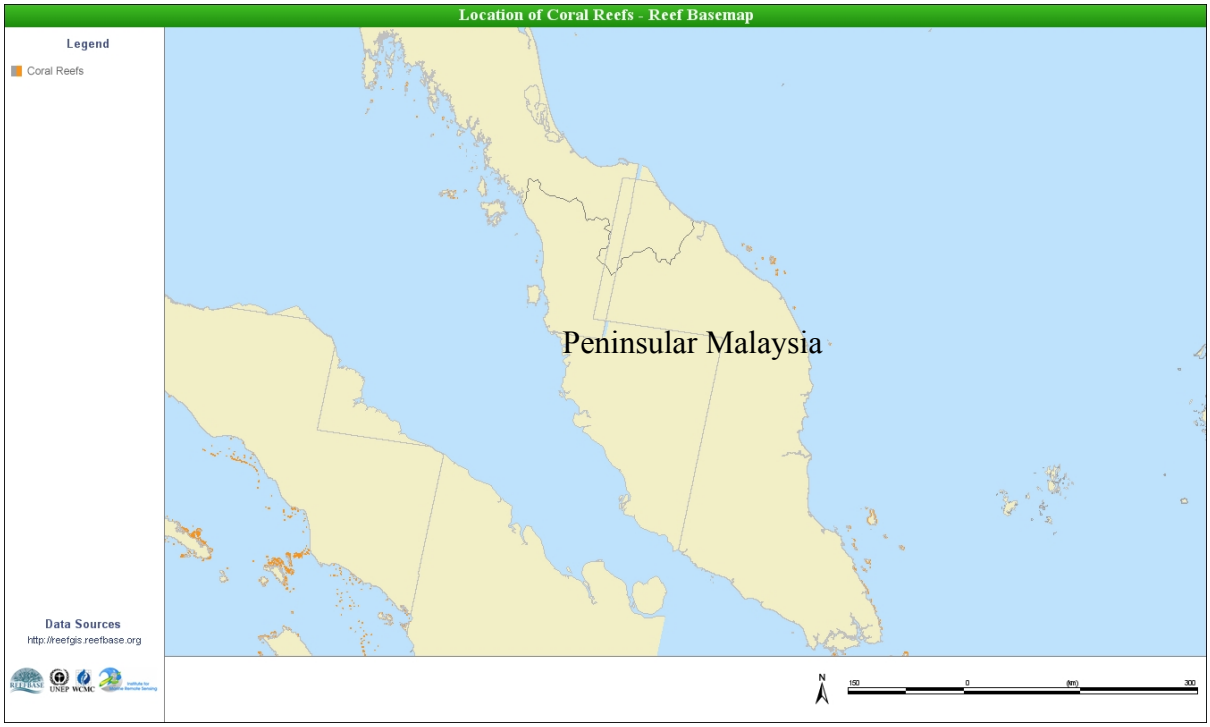


Figure 1.3: Location of coral reefs in Peninsular Malaysia (Longitude 100.113 Latitude 6.428) (reefGIS.reefbase.org)

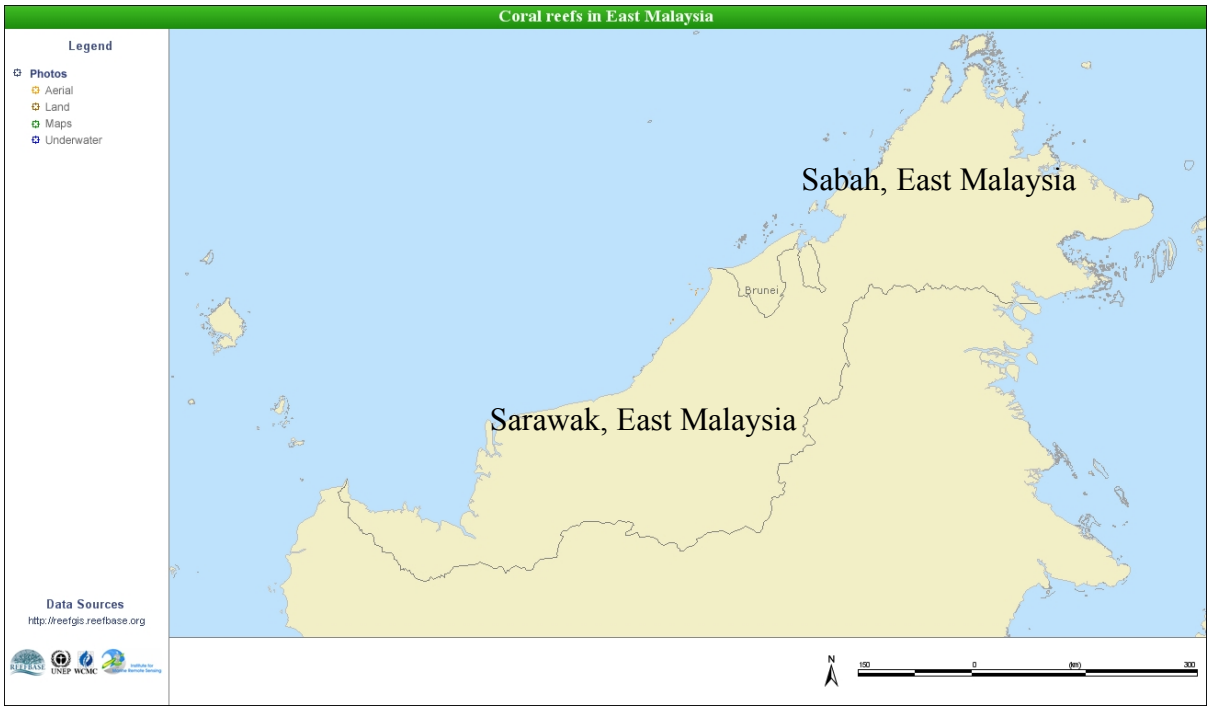


Figure 1.4: Location of coral reefs in East Malaysia (Longitude 104.301 Latitude 1.345) (reefGIS.reefbase.org)

Malaysian corals were affected during the global mass bleaching events of 1998 and 2010 resulting in many bleached corals some of which recovered, but coral degradation followed. Corals in Peninsular Malaysia and Eastern Malaysia were also degraded by sedimentation and effluent discharges from the tourism areas (UNEP, 2007). Untreated sewage flows directly into the ocean from the hotels, resorts and chalets in Redang, Tioman and Sibu-Tinggi Islands (UNEP, 2007). Nutrient runoff from land use was reported as a significant threat to reefs in marine parks all over Malaysia (Harborne et al., 2000), but at this time, Malaysian coral cover was reported as in 'good' condition (52.8%) based on the ASEAN-Australia Living Coastal Resources project, even though it has been reduced since the pre-1998 bleaching event (Harborne et al., 1998).

Reef Check Malaysia has undertaken Reef Check survey monitoring on Malaysian coral health since 2007 on several islands in Peninsular Malaysia; Tioman, Perhentian and Redang Island (Hyde et al., 2013). By this methodology, reefs are monitored on a regular basis and any changes may be detected providing warnings of environmental events or progressive degradation (Hyde et al., 2013). This could give researchers and managers in Malaysia information to initiate coral reef damage management actions, before the reefs become more deteriorated (Hyde et al., 2013). As mentioned by Tun et al. (2004), Reef Check methods had been applied in most countries in South East Asia for coral reef monitoring. Waheed et al. (2015) recorded the hard coral species richness and benthic reef assemblages by following the Reef Check substrate categories, and this study initiated conservation zoning plans for a protected marine park. A similar coral survey was also conducted at Tun Sakaran Marine Park, Sabah (East Coast of Malaysia), and reported coral decline and algal competition as a result of observations of high densities of *Stegastes* damselfish (more than 200 per $100m^2$) (Montagne

et al., 2013). Reef Check surveys identify 'Indicator Species' to determine the coral's health *in situ* (Hyde et al., 2013).

Between 2010 and 2012, Reef Check Malaysia (RCM) completed a series of reef rehabilitations on Pangkor, Tioman and Redang Islands, Peninsular Malaysia, as one of the initiatives to conserve coral reefs (Hyde et al., 2013). Reef rehabilitation programs are one conservation initiative to slow the declination rates, and manage the recovery of coral cover and its surrounding area (Hyde et al., 2013).

During the global bleaching event in 1997/1998, bleaching in Malaysian waters was mild and only seen in parts of Sabah, East Coast of Malaysia (Wilkinson, 1998). 40% of coral colonies in Layang-layang Island, Sabah were bleached in the 1998 event, but eventually recovered in 1999 (Waheed et al., 2015). In May 1998, 30-40% of corals in Pulau Gaya, Sabah, were bleached at seawater temperature of 32°C (Pilcher and Cabanban, 2000). Overall, it is reported that an increased temperature of 2-3°C above normal sea surface temperature was the cause of the mass bleaching event (UNEP, 2007). Bleaching in Payar Island, Langkawi, West Peninsular Malaysia, was first observed in 1995 (Jonsson, 2003).

However, in 2010, during the El Nino event, half of the coral communities in Tioman, Redang and Perhentian Islands were bleached and two-thirds were reported completely white (Tan and Heron, 2011). 7.28% corals in Teluk Nyior (Park of Langkawi island) were recorded bleached in 2010, but some recovered in 2011 and 2012 (BOBLME, 2014). There were less than 10% of bleached corals found in parts of Eastern Malaysia during the 2010 ENSO (Tan and Heron, 2011). Guest et al., (2012) reported that during the 1998 bleaching event, Malaysian *Acropora* and *Pocillopora* were unbleached but massive taxa such as *Diploastrea*, massive *Porites*,

Montastraea and *Goniastrea* were found to be badly bleached. This in contrast with the hierarchy of taxa susceptibility (*Acropora* > bleaching susceptibility than *Porites* and faviids) (Guest et al., 2012). Due to this event, the Malaysian government took an action of closing all Marine Park dive sites where damaged by more than 60% (Thomas and Heron, 2011) for the corals to regenerate during recovery time (AFP, 2010).

1.8.1 Threats to corals in Malaysia

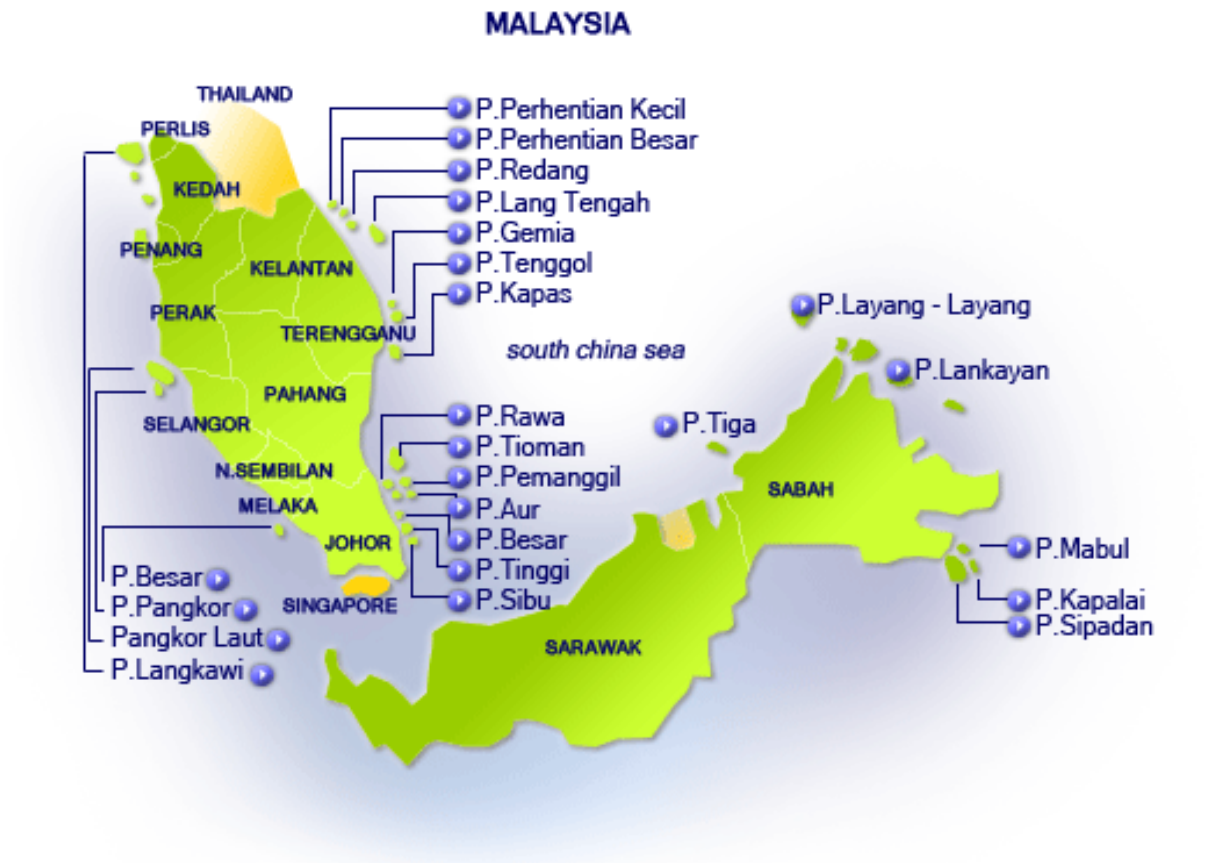


Figure 1.5: Location of islands in Malaysia

In Peninsular Malaysia, coral reef threats are development and tourism related; land-based pollution, sewage pollution and boats, anchors impact (Reef Check Malaysia, 2016). Reef-related tourism in Perhentian Island is valued at RM120 million, and threatened by the spread of Nutrient Indicator Algae (NIA) on the reefs, caused by sewage pollution (Reef Check Malaysia, 2012). Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) from water samples of the marine park, prove that the marine park is polluted with sewage effluent (Reef Check Malaysia, 2012). In 1984, Malaysian creek waters had concentrations of particulate organic nitrogen (PON), ranging from <10 to 131 µM were found while dissolved organic nitrogen (DON) were ranging between 20-50 µM (Nixon et al., 1984). Tioman water

quality was found to contain high levels of coliform bacteria, indicated sewage pollution as well (Reef Check Malaysia, 2012). Poor sewage infrastructure and inadequate sewage treatment systems are the cause of similar issues of high NIA levels in Perhentian, Redang, Tioman and Tenggol marine parks, Peninsular Malaysia (Reef Check Malaysia, 2012). Redang Island waters were also to be found with sewage pollutants during surveys in 1995 to 2000 (Law et al., 2001). Sewage polluted marine water of Sibu-Tinggi Island in west coast of Peninsular Malaysia was because of an undeveloped sewage treatment system (UNEP, 2007). It was suggested to improve the existing systems and invest in a mobile septic tank de-sludging facility to counter the sewage pollution problems in the problematic areas (Reef Check Malaysia, 2012). In 2003, studies found bleached corals in Payar Island Marine Park, Langkawi (west coast of Peninsular Malaysia), caused by high levels of phosphate and nitrate, from the sewage pollution (Jonsson, 2003). Other than that, 50-70% corals in Langkawi were found to be covered with sediment and almost 100% of dead corals were sediment-covered (Jonsson, 2003). The sedimentation was believed to be from the coastal area construction for a new tourism destination (Jonsson, 2003). Reefs at Risk in Southeast Asia recorded that 23% of Malaysian corals were affected by coastal development and sedimentation (UNEP, 2007).

In Sarawak, Eastern Malaysia, the reefs in the Miri area are threatened with sedimentations and nutrient enrichment from sand mining activity from many rivers for the last three decades (Pilcher and Cabanban, 2000). While in Sabah, tropical storm Greg in 1996 was the cause of coral cover loss in Tunku Abdul Rahman Park (TARP), besides anthropogenic effects such as fish bombing and pollution. Corals in TARP were also being affected by sedimentation and nutrient-enrichment from the water draining in nearby town, Kota Kinabalu Bay (Pilcher and Cabanban, 2000). Over-fishing and destructive fishing and also, the lack of MPA management,

are also the threats to coral reefs in East Malaysia. Reefs at Risk mentioned that 68% of Malaysian corals were affected by blast and poison fishing (UNEP, 2007).

To date, Malaysian reefs exhibit a relatively high level for living coral (45.95%), degraded from 2014 surveys which recorded 48.10% (Reef Check Malaysia, 2016). This percentage is in 'fair' condition, based on the Coral Reef Health Criteria, developed by Chou et al. (1994), as shown in Table 1.2. The Nutrient Indicator Algae (NIA) recorded by Reef Check Malaysia (2016) was critical in parts of Sabah, with more than 30% algae domination amongst corals. This condition can cause algae-dominated-reefs, meaning an unproductive coral reef ecosystem (Reef Check Malaysia, 2016). This survey was done on several islands off Peninsular Malaysia, covering both Marine Protected Areas and non-protected areas, and in various parts of Sabah and Sarawak, East Malaysia waters.

Table 1.2: Coral Reef Health Criteria

Percentage of live coral cover	Rating
0-25	Poor
26-50	Fair
51-75	Good
76-100	Excellent

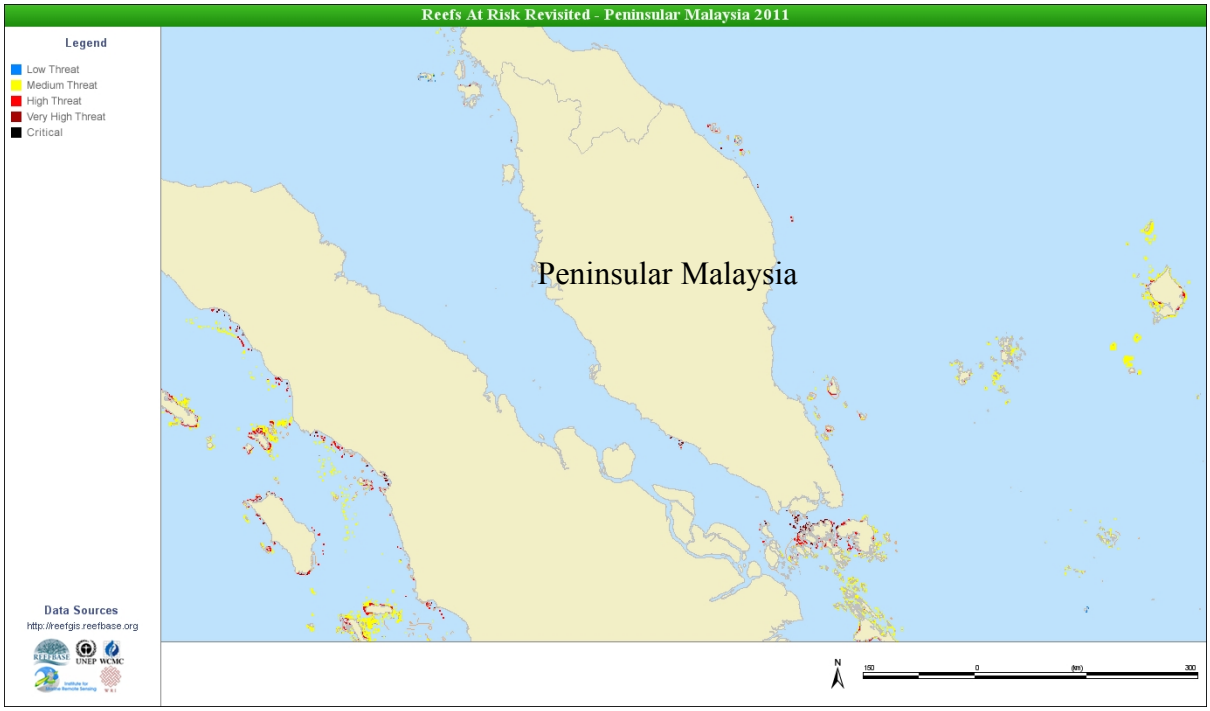


Figure 1.6: Coral reef at risk in Peninsular Malaysia in 2011 (Longitude 100.113 Latitude 6.428) (reefGIS.reefbase.org)

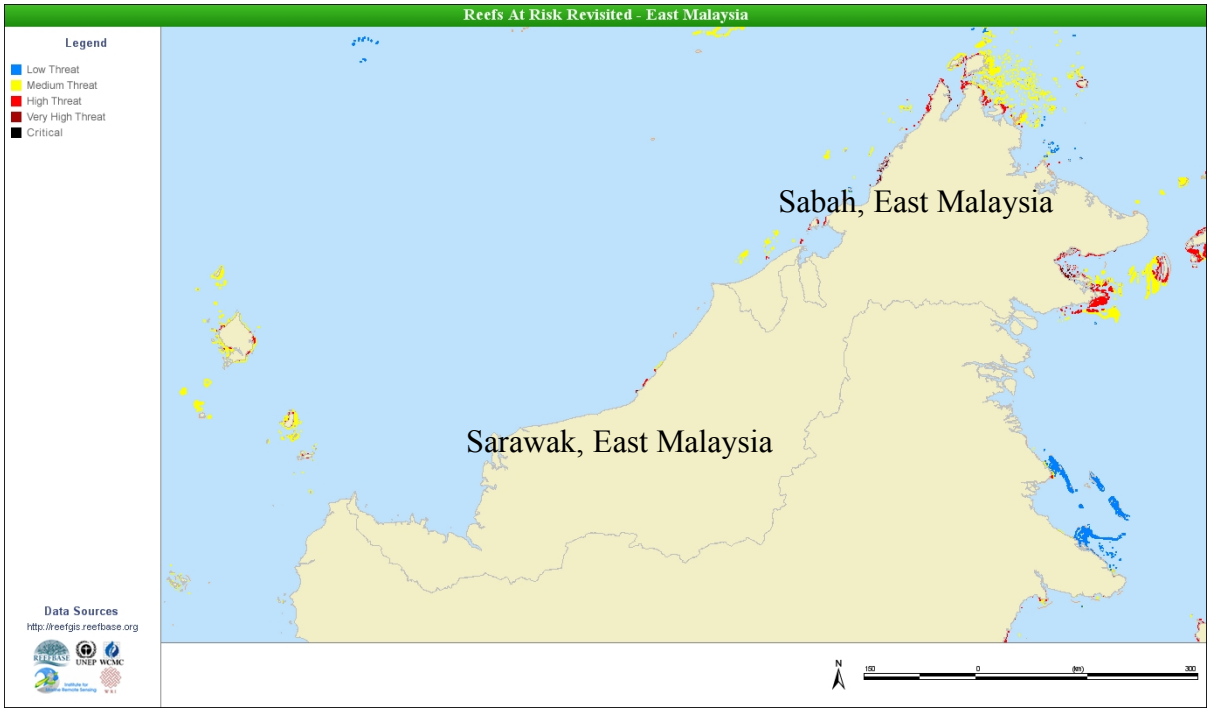


Figure 1.7: Coral reef at risk in East Malaysia in 2011 (Longitude 104.301 Latitude 1.345) (reefGIS.reefbase.org)

1.8.2 Coral management in Malaysia

The Department of Marine Parks Malaysia (DMPM) has been responsible for the management of marine resources for over 20 years in Peninsular Malaysia (Reef Check Malaysia, 2016). Lack of awareness and information among the stakeholders are the weaknesses of the current coral management practices in Peninsular Malaysia (Reef Check Malaysia, 2016). In Perhentian Island specifically, the stakeholders; Besut District Department of Marine Parks (Terengganu), entrepreneurs and tourists agreed on coral reef conservation through education, awareness campaign, MPA protection and media co-operation for news (Saleh and Hasan, 2014). MPAs can help build coral reef resilience by supporting and enhancing factors of good coral reef condition, biological diversity, connectivity and favorable local conditions (Marshalls et al., 2006). Coral reef conservation and resources in Sabah state, Eastern Malaysia is under the management of the Department of Sabah Fisheries, Sabah Parks and the Sabah Ministry of Science, Tourism and Environment (Pilcher and Cabanban, 2000). Its conservation management is limited by lack of trained personnel and low funds for MPA regulation enforcement (Pilcher and Cabanban, 2000). Most countries in South East Asia are having problems due to insufficient training for coral reef monitoring, with a range of 10-60 people in every country (Tun et al., 2004). Malaysia needs to develop a coral reef database and management data system of coral reef monitoring. This is because, there are many agencies, organisations and institutions involved in data archival and analysis of coral reefs in Peninsular Malaysia and East Malaysia, but no centralized data coordination (Tun et al., 2004). A coral reef database for Malaysia would provide a background status to help access information for emergencies such as coral bleaching events, post-tsunami and for assisting coral reef management (Tun et al., 2005). Artificial reef projects, coral transplantation and coral culturing

are among the efforts that have been made to conserve Malaysian reefs by many institutions, including the Department of Fisheries Malaysia and corporate bodies (UNEP, 2007).

1.9 Scope of the thesis

This study aims to assess how corals common to Malaysia respond to environmental stress, in particular elevated temperature, irradiance and nutrients, which are common factors affecting corals around the nation's coasts. These factors often occur in combination and therefore the synergistic effects will be examined.

This study was guided by the overacrching hypothesis: Reefs in Malaysia have degraded due to direct human impacts combined with global warming events. Live coral cover in all islands in Malaysia is reducing because of synergistic effects of anthropogenic and climate change factors. The reefs have shown only slow recovery from the 2010 bleaching event because there are continuing problems with coastal development, tourism, pollution from sewage activities and agricultural activities. Measured by widely used coral reef health criteria (Table 1.2), reefs in Malaysia are in poor to fair condition, rather than fair to good condition to date, because of the on-going anthropogenic impacts and uncontrollable global threats (climate change events). There is need to build resilience in coral reefs, that is, the biological ability of the coral reefs to recover from natural and human stresses. By knowing the resilience of coral species, and by identifying resilient areas of reef, a management strategy can be build to contribute to future coral reef recovery.

The main objective of this thesis is to provide baseline information on how environmental change may affect Malaysian coral species and to provide an understanding of the resilience of future coral populations. The goal was to examine the performance of a selection of typical

corals from the region through their physiological parameters after synergistic stress. Physiological parameters such as photochemical efficiency can indicate the key responses of corals to stresses. Each coral species responds differently towards stresses, therefore there are corals that can overcome the environment disturbances. Reef check data were also to look into coral bleaching stressors in terms of real recorded data. Reef Check data were related with 50-km monitoring product of Degree Heating Week Coral Reef Watch for 2009 until 2011 in Peninsular Malaysia and East Malaysia.

References

Abdul-Adzis K.A., Amri A.Y., Oliver J. and Mohamed C.A.R. 2009. Effective and maximum quantum yield of the lace coral *Pocillopora damicornis* (Anthozoa: Scleractinia: Pocilloporidae) in Pulau Tioman, Malaysia. *Journal of Science and Technology in the Tropics* **5**: 13-17.

Adir N., Zer H., Schochat S. and Ohad I. 2003. Photoinhibition – a historical perspective*. *Photosynthesis Research* **76**: 343-370.

Anthony K.R.N., Conolly S.R. and Hoegh-Guldberg O. 2007. Bleaching, energetic and coral mortality risk: Effects of temperature, light and sediment regime. *Limnology Oceanography* **52** (2): 716-726.

Badgley B.D., Lipschultz F. and Sebens K.P. 2005. Nitrake uptake by the reef coral *Diploria strigose*: effects of concentration, water flow and irradiance. *Marine Biology* **149**: 327-338.

Baohua Z., Guangce W., Bo H. and Tseng C.K. 2004. Effects of temperature, hypoxia, ammonia and nitrate on the bleaching among three coral species. *Chinese Science Bulletin* **49** (2): 1923-1928.

Béraud E., Gevaert F., Rottier C. and Ferrier-Pagès. 2013. The response of the scleractinian coral *Turbinaria reniformis* to thermal stress depends on the nitrogen status of the coral holobiant. *The Journal of Experimental Biology* **216**: 2665-2674.

Bhagooli R. and Hidaka M. 2004. Photoinhibition, bleaching susceptibility and mortality in two scleractinian corals, *Platygyra ryukyuensis* and *Stylophora pistillata*, in response to thermal and light stresses. *Comparative Biochemistry and Physiology Part A* **137**: 547-555.

Birkeland C. 1996. *Life and Death of Coral Reefs*. United States of America: Chapmen & Hall Book.

Borneman E.H. 2009. *Aquarium corals: selection, husbandry and natural history*. 3rd ed. United States of America: T.F.H. Publications.

Burke L., Selig L. and Spalding M. 2002. *Reefs at risk in Southeast Asia*. Cambridge: UNEP-WCMC.

Burke L., Reytar K., Spalding M. and Perry A. 2011. *Reefs at risk revisited*. Washington: World Resources Institute.

Coffroth, M.A., Santos, S.R., 2005. Genetic diversity of symbiotic dinoflagellates in the genus Symbiodinium. Protist **156** (19–34).

Coles C. 2007. Coral health and water quality evaluations at Waiopae tide pools, Kapoho, Big Islands of Hawai'i. *Hohonu Journal* **5** (1).

De'ath G. and Fabricius K. 2010. Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. *Ecological Applications* **20** (3): 840-850.

Douglas A.E. 2003. Coral Bleaching – how and why. *Marine Pollution Bulletin* **46**: 385-392.

Dubinsky and Stambler 1996. Marine pollution and coral reefs. *Global Change Biology* **2:** 511-526.

Fabricius, K. E. 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Marine Pollution Bulletin* **50**: 125-146.

Faxneld S., Jörgensen T.L., Tedengren M. 2010. Effects of Elevated Water Temperature, Reduced Salinity and Nutrient Enrichment on the Metabolism of the coral *Turbinaria mesenterina*. *Estuarine, Coastal and Shelf Science* **88**: 482-487.

Franklin L.A., Seaton G.G.R., Lovelock C.E. and Larkum A.W.D. 1996. Photoinhibition of photosynthesis on a coral reef. *Plant, Cell and Environment* **19**: 825-836.

Garbunov M.Y., Kolber Z.S., Lesser M.P. and Falkowski P.G. 2001. Photosynthesis and photoprotection in symbiotic corals. *Limnology and Oceanography* **46**: 1.

Glynn P.W. 1991. Coral reef bleaching in the 1980s and possible connections with global warming. *Trends in Ecology and Evolution* **6**: 175-179.

Goodson M.S., Whitehead L.F. and Douglas A.E. 2001. Symbiotic Dinoflagellates in Marine Cnidaria: Diversity and Function. *Hydrobiologia* **461**: 79-82.

Gustafsson M.S.M., Baird M.E. and Ralph P.J. 2013. The Interchangeability of Autotrophic and Heterotrophic Nitrogen Sources in Scleractinian Coral Symbiotic Relationships: A Numerical Study. *Ecological Modelling* **250**: 183-194.

Harborne A., Fenner D., Barnes A., Beger M., Harding S. and Roxburgh T. 2000. Status report on the coral reefs of the east coast of Peninsula Malaysia. *Coral Cay Conservation Ltd*.

Hanelt D. 1998 Capability of dynamic photoinhibition in Artic macroalgae is related to their depth distribution. Capbability of dynamic photoinhibition in Artic macroalgae is related to their depth distribuition. *Marine Biology* **131**: 361-369.

Hoegh-Guldber O. 1999. Climate Change, Coral Bleaching and the Future of the World's Coral Reefs. *Marine and Freshwater Research* **50**: 839-866.

Hoegh-Guldberg O. and Jones R.J. 1999. Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. *Marine Ecology Progress Series* **183**: 73-86.

Hodgson G. 1999. A global assessment of human effects on coral reefs. *Marine Pollution Bulletin* **38** (5): 345-355.

Hoogenboom M.O., Anthony K.R.N. and Conolly S.R. 2006. Energetic cost of photoinhibition in corals. *Marine Ecology Progress Series* **313**: 1-12.

Jackson A.E., Miller D.J. and Yellowlees D.1989. Phosphorus metabolism in the coral-zooxanthellae symbiosis: characterization and possible roles of two acid phosphatases in the algal symbiont *Symbiodinium* sp. *Proceedings of the Royal Society of London* **B 238**: 193-202.

Jones R.J., Hoegh-Guldberg O., Larkum A.W.D. and Schreiber U. 1998. Temperature-induced bleaching of corals begins with impairment of the CO2 fixation mechanism in zooxanthellae. *Plant, Cell and Environment* **21** (12): 1219-1230.

Jones R.J., Ward S., Yang Amri A. and Hoegh-Guldberg O. 2000. Changes in quantum efficiency of Photosystem II of symbiotic dinoflagellates of corals after heat stress, and of bleached corals sampled after the 1998 Great Barrier Reef mass bleaching event. *Marine Freshwater Resources* **51:** 63-71.

Jones A.M., Berkelmans R., van Oppen M.J.H., Mieog J.C. and Sinclair W. 2008. A Community Change in the Algal Endosymbionts of a Scleractinian Coral Following a Natural Bleaching Event: Field Evidence of Acclimation. *Proceedings of The Royal Society London* **275**: 1359-1365.

Kok B. 1956. On the Inhibition of Photosynthesis by Intense Light. *Biochim Biophys Acta* **21**: 234-244.

Koop K., Booth D., Broadbent A., Brodie J., Bucher D., Capone D., Coll J., Dennison W., Erdmann M., Harrison P., Hoegh-Guldberg O., Hutchings P., Jones G.B., Larkum W.D., O'Neil J., Steven A., Tentori E., Ward S., Williamson J. and Yellowlees D. 2001. ENCORE. The Effect of Nutrient Enrichment on Coral Reefs. Synthesis of Results and Conclusions. *Marine Pollution Bulletin* **42** (2): 91-120.

Lesser M.P. 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. *Limnology Oceanography* **41** (12): 271-283.

Mazlan A.G., Zaidi C.C., Wan-Lofti W.M. and Othman B.H.R. 2005. On the current status of coastal marine biodiversity in Malaysia. *Indian Journal of Marine Sciences* **34** (1): 76-87.

Marubini F. and Davies P.S. 1996. Nitrate Increases Zooxanthellae Population Density and Reduces Skeletogenesis in Corals. *Marine Biology.* **127**: 319-328.

Mieog J.C., Olsen J.L., Berkelmans R., A. Bleuler-Martinez S., Willis B.L. and J.H. Van Oppen Madeleine. 2009. The roles and interactions of symbiont, host and environment in defining coral fitness. *PLoS One* **4**(8).

Morelock J., Boulon K. and Galler G. 1979. Sediment Stress and Coral Reefs. *Symposium of Energy Industry and the Marine Environment in Guayanilla Bay,* Ed. J. M. Lopez. 46-58.

Murata N., Takahashi S., Nishiyama Y., and Allakhverdiev S.I. 2007. Photoinhibition of Photosystem II Under Environmental Stress. *Biochimica et Biophysica Acta* **1767**: 414-421.

Muscatine L., Falkowski P.G., Dubinsky Z., Cook P.A. and McCloskey L.R. 1989. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. *Proceedings of Royal Society London* **B 236**: 311-324.

Osmond C.B. 1994. What is Photoinhibition: Some Insights from Comparisons of Sun and Shade Plants. *In Photoinhibition of Photosynthesis: from Molecular Mechanisms to the Field*. BIOS Scientific Publishers, Oxford. Pp 95-110.

Osmond B. and Förster B. 2008. Photoinhibition: Then and Now. In Photoprotection, Photoinhibition, Gene Regulation and Environment. *Springer, The Netherlands*. pp. 11-22.

Parsons M.L., Walsh W.J., Settlemier C.J., White D.J., Ballauer J.M., Ayotte P.M., Osada K.M. and Carman B. A multivariate assessment of the coral ecosystem health of two embayments on the lee of the island of Hawai'i. *Marine Pollution Bulletin*.

Pastore M., 2014. The impact of nitrogen eutrophication on Caribbean coral reefs: A review. *Concept* **Vol. XXXVII.**

Praveena S.M., Siraj S.S. and Aris A.Z. 2012. Coral reef studies and threats in Malaysia: a mini review. *Rev Environmen Sci Biotechnol* **11**: 27-39.

Reef Check Malaysia Annual Survey Report. 2010. Reef check Malaysia, Kuala Lumpur.

Reef Check Malaysia Annual Survey Report. 2014. Reef check Malaysia, Kuala Lumpur.

Renegar D.A. and Riegl B.M. 2005. Effect of nutrient enrichment and elevated CO2 partial pressure on growth rate of Atlantic scleractinian coral *Acropora cervicornis. Marine Ecology Program Series* **293**: 69-76.

Rowan R. 1998. Diversity and ecology of zooxanthellae on coral reefs. *Journal of Psychology* **34**: 407-417.

Salih A., Hoegh-Guldberg O. And Cox G. 2006. Bleaching Responses of Symbiotic Dinoflagellates in Corals: The Effects of Light and Elevated Temperature on their Morphology and Physiology. In: Greenwood, J.G. & Hall, N.J., eds (1998). *Proceedings of the Australian Coral Reef Society 75th Anniversary Conference, Heron Island October 1997*. pp. 199-216.

Shahbudin S., Hafiz H.Z., John B.A., Kamaruzzaman B.Y. and Jalal K.C.A. 2011. Distribution and diversity of corals on artificial reefs at Pasir Akar and Teluk Kalong, Redang Island, Malaysia. *Journal of Applied Sciences* **11** (2): 379-383.

Silverstein R.N., Correa A.M.S. and Baker A.C. 2012. Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. *Proceedings of the Royal Society Biological Sciences.*

Smith D.J., Suggett D.J. and Baker N.R. 2005. Is photoinhibition of zooxanthellae photosynthesis the primary cause of thermal bleaching in corals. *Global Change Biology* **11** (1): 1-11.

Sonoike K. 1996. Photoinhibition of Photosystem I: Its physiological significance in the chilling sensitivity of plants. *Plant Cell Physiology* **37** (3): 239-247.

Stat M., Morris E. and Gates R.D. 2008. Functional diversity in coral-dinoflagellate symbiosis. *PNAS* **105** (27): 9256-9261.

Stimson J. and Kinzie III R.A. 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. *Journal of Experimental Marine Biology* **153** (1991): 63-74.

Stimson J., Sakai K. and Sembali H. 2002. Interspecific Comparison of the Symbiotic Relationship in Coral with High and Low Rates of Bleaching-Induced Mortality. *Coral Reefs* **21**: 409-421.

Sugget D.J. and Smith D.J. 2009. Interpreting the sign of coral bleaching as friend vs. foe. *Global Change Biology* **17** (1): 45-55.

Toda T., Okashita T., Maekawa M., Alfian A.A., Rajuddin M.K., Nakajima R., Chen W., Takahashi K.T., Othman H.R. and Tera-Zaki M. 2007. Community structures of coral reefs around Peninsular Malaysia. *Journal of Oceanography* **63**: 113–123.

Tomascik T. and Sander F. 1987. Effects of eutrophication on reef-building corals. III Reproduction of the reef-building coral *Porites*. *Marine Biology* **94**: 77-94.

Venn A.A., Loram J.E. and Douglas A.E. 2007. Photosynthetic Symbioses in Animals. *Journal of Experimental Botany*.

Wear S.L. and Thurber R.V. 2015. Sewage pollution: mitigation is key for coral reef stewardship. Annals of the New York Academy of Sciences, 1355 (1): 15-30.

Webb K.L. 1975. Enewetak (Eniwetok) Atoll: aspects of the nitrogen cycle on a coral reef. *Limnology Oceanography.* **20**: 198-210.

Weis V.M. 2008. Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *The Journal of Experimental Biology* **211**: 3059-3066.

Wilkinson C.R. 1999. Global and Local Threats to Coral Reef Functioning and Existience: Review and Predictions. *Marine and Freshwater Research* **50**: 867-878.

Wilkinson C.R. 2008. Status of coral reefs of the world. Townsville: *Global Coral Reef Monitoring Network and Reef and Rainforest Research Center.*

Winter G., Loya Y., Röttgers R. and Beer S. Photoinhibition in shallow-water colonies of the coral *Stylophora pistillata* as measured *in situ. Limnology Oceanography* **48** (4): 1388-1393.

Wooldridge S.A. and Done T.J. 2009. Improved water quality can ameliorate effects of climate change on corals. *Ecological Applications* **19**(6): 1492-1499.

Yee S.H., Santavy D.L. and Barron M.G. 2008. Comparing Environmental Influences on Coral Bleaching Across and Within Species Using Clustered Binomial Regression. *Ecological Modelling* **218**: 162-174.

Yonge C.M. and Nicholls A.G. 1931. Studies on the physiology of corals: IV. The structure, distribution and physiology of the zooxanthellae. In *Scientific Reports/Great Barrier Reef Expedition 1928-1929* **Vol. 1:** 135-176.

Chapter 2

PAM Fluorometer Techniques and Coral Health

2.1 Introduction

After the bleaching events of Malaysian reefs of 1998 and 2010, the Department of Marine Parks and various non-government organizations in Malaysia were alert to factors that can predict coral bleaching such as sea surface water temperature, from bleaching alerts (NOAA, 2017) and Reef Check field monitoring, where reef changes can be tracked by time (Chelliah et al., 2014). However, according to Reef Check Malaysia (2008), Malaysia still lacks coral reef management programs because of a paucity of information on the status and location of reefs.

Thus, this investigation is to study the photosynthetic performance of corals and the effects of stress to help predict the risk of mortality from coral bleaching events. The aim is to provide a baseline of the yield values in its 'normal' state and after-stress state which can be used to monitor the health of the specific coral species in the natural environment. This will be achieved by estimating the maximum quantum yield of three common Malaysian coral species and recording their physical changes (paling and mortality) after temperature-irradiance stress treatments and temperature-nitrate stress treatments. An outcome of this study is to understand the maximum quantum yield of stressed corals in specific species and to produce an index of quantum yield of healthy and bleached corals in Malaysia generally. By collecting this quantum yield data, it will help researchers to understand the condition of some coral species in bleaching events caused by temperature, light or nitrate stress in Malaysia. In Malaysia, there is little documentation of maximum quantum yield of dark-adapted coral species, especially in coral bleaching data (Abdul-Adzis et al., 2009). Photosynthetic performance will be assessed using a

Pulse Amplitude Modulated (PAM) Flurometer, where photochemical efficiency is measured. This chapter explains the background of chlorophyll fluorescence and PAM fluorometer methodology.

2.2 Chlorophyll Fluorescence

Chlorophyll fluorescence is an indicator of photosynthetic energy conversion in higher plants, algae and bacteria (Consalvey et al. 2005). To gain detailed information on the state of photosystem II (PSII), chlorophyll fluorometry is one of the most popular techniques in plant physiology (Murchie and Lawson, 2013). Chlorophyll fluorescence arising from the chloroplast thylakoid membranes exemplifies the primary processes of photosynthesis, such as light absorption, excitation energy transfers and the photochemical reaction in PSII (Krauss and Weis, 1988).

Fluorescence is the re-emission of energy in the form of a photon (light) as an electron returns to ground state from a single excited state (Cosgrove and Borowitzka, 2011). When the chlorophyll molecule absorbs different wavelengths of light, it transforms energy by charge separation, heat dissipation or resonance energy transfer (electron drops out of the excited state) (Cosgrove and Borowitzka, 2011). In 1966, biological oceanography studies first used the technique of chlorophyll fluorescence (Cosgrove and Borowitzka, 2011). Chlorophyll fluorescence analysis is sensitive, non-invasive and a simple tool for studying photosynthesis, biophysics, biochemistry and green plants physiology (Misra et al., 2012). There are five types of fluorometers to measure chl-*a* fluorescence, depends on the type of study and suitability of the photosynthetic systems. The types of fluorometer and its functions are shown in Table 2.1.

Table 2.1: Types of fluorometer to measure chlorophyll *a* fluorescence depending on the type of study and suitability of the photosynthetic systems.

Type	Function	Instrument
Pulse Amplitude Modulation Fluorometer (PAM)	- Stress assessment studies with marine plants and algae - Assess photochemical efficiency of photosystem II (PSII) of zooxanthellae (Jones et al., 1999).	Water-PAM, Diving-PAM, MINI-PAM
Fast Repetition Rate Fluorometer (FRR)	- Variable fluorescence characteristics of phytoplankton - Evaluate primary productivity - Determines photosynthetic efficiency of phytoplankton samples (F_v/F_m), to measure the phytoplankton's health (Raateoja, 2004)	
Fluorescence Induction and Relaxation System (FIRe)	- To measure variable chlorophyll fluorescence in photosynthetic organisms - Based on the Fast Repetition Rate Fluorometry (FRRF) technique - It has been simplified on the electronic circuitries and measurement, improved optical design - More sensitive, reliable and the production cost was reduced. (Garbunov and Falkowski, 2004)	
Pump and Probe Fluorometer (PandP)	- Active fluorescence methods use an artificial light source to activate chlorophyll (*Chl*) fluorescence.	

	- Quantum yield of fluorescence changes can be controlled, measured with a weak probe flash, is induced by an actinic (pump) flash. - *in-situ* technique (Kolber and Falkowski, 1993) - Can be used in a different levels of plant's trophic level, as well as in climatic and hydrophysical characteristics (Antal et al., 2001).
Induction Fluorometer/ContinousExcitation Fluorometer	- A continuous excitation fluorimeter is designed to measure the Kautsky Induction or Fast Chlorophyll Fluorescence Induction (Kautsky and Hirsch, 1931)

The principle of Pulse-Amplitude-Modulation (PAM) is the fluorescence yield which depends on the light conditions, gives a measure of photosynthesis process (Schreiber, 2004). The PAM fluorometer is based on the technique of quenching analysis of modulated fluorescence by the saturation pulse (Misra et al., 2012). It is a non-intrusive methodology for measuring fluorescence yield in all physiologically conditions without damaging the plant sample (Schreiber, 2004). PAM fluorometer has its own characteristics based on Schreiber (2004):

a) For a dark-adapted sample (sample put in dark container for certain time in minutes), the measuring light must be in a low state.
b) For assessment of maximal fluorescence yield, the PAM fluorometer can detect the high fluorescence excitement from the measuring light or the natural lighting, with a suitable optical filter.
c) It can give quick response to rapid changed in fluorescence yield of the sample during dark-light and light-dark transitions.

PAM fluorometry is used to measure the relative quantum yield of chlorophyll fluorescence by applying light with constant saturation pulse amplitude (Schreiber, 2004). The quantum yield of photosynthesis is a molar ratio between oxygen released or carbon assimilated to photons absorbed in the process (Fig. 2.1) (Iluz and Dubinsky, 2013). When the instrument excited a light pulse, after the probe is put on the sample, the absorbed light energy will re-emit as fluorescence (Schreiber, 2004). PAM fluorometry is used for chlorophyll fluorescence measurements, in a process in which the chlorophyll molecules absorb the light energy for photochemistry or where excess energy is lost as heat or re-emitted as light; an increase in one process will cause a decrease in other process (Maxwell and Johnsson, 2000). To measure chlorophyll fluorescence yield, the sample must be exposed to pulse-amplitude light of defined wavelength and an amount of re-emitted light at longer wavelengths will be measured (Maxwell and Johnsson, 2000).

In a dark-adapted sample illuminated with continuous light, the chlorophyll fluorescence intensity will show changes, which are known as: fluorescence induction, fluorescence transient, or Kautsky effect (Govindjee, 1995). The dark adapted technique is where a sample of plant must be dark adapted to avoid ambient light interference (Fernandez-Jaramillo et al., 2012). In this situation (dark-adapted sample illuminated with light), the fluorescence yield will increase within seconds (<10-15 s^{-1}) from **minimal measurement (F_o)** to a **maximum measurement (F_m).** The differences between these yields is called **various fluorescence (F_v)** (Screiber, 2004; Misra et al., 2012) and it is used as the maximum quantum yield of primary PSII photochemistry. The fluorescence yield increment is a consequence of reduction of electron receptors in the photosynthetic pathway, downstream of PSII (plastoquinone and $\mathbf{Q_A}$) (Maxwell and Jonsson, 2000). PSII contains pigments and proteins in the thylakoid membrane of chloroplasts which can show its sensitivity towards any stresses (Fernandez-Jaramillo et al., 2012). PSII is the most

vulnerable part of the photosynthetic system to light-induced damage (Maxwell and Johnson, 2000). When the sample is moved from a dark adaption into light, the fluorescence intensity will be slowly decreased to avoid the adverse effects of excess light (Maxwell and Johnson, 2000). The reaction centre of PSII is said to be 'closed', and the efficiency of photochemistry is reduced (Maxwell and Johnson, 2000). This condition is known as **non-photochemical quenching**, which can cause the photoinhibition process (Misra et al., 2012). In a laboratory, a dark-adapted value of F_m can be measured by full-night and 24h of dark adaptation, while in a field experiments, pre-dawn value of F_m can be used as a reference point (Maxwell and Johnson, 2000). All the terms for chlorophyll fluorescence parameters were summarized in Table 2.2.

The process whereby the electrons are transferred from the reaction centre of PSII to Q_A quenches fluorescence, is known as **photochemical quenching** (Baker, 2008). When the non-photochemical mechanism functions, it is attributed to enhance thermal dissipation in the PSII reaction centres themselves. This may lead to the reaction centre temporarily ceasing to operate but it is not actually damaged (Garbunov et al., 2001). In photochemical quenching, Q_A is oxidized, thus it accepts the electrons from the photosystem II reaction centre and the electrons will pass along the photosynthetic electron transport chain (Jones et al., 1999). Because of that, the process will produce oxidation of water, oxygen evolution, reduction of $NADPH^+$ to NADPH, membrane proton transport, ATP synthesis and reduction of carbon dioxide to carbohydrate in the dark reactions of photosynthesis. In a situation where a photosynthetic symbiotic animals or plants were given a high intensity and short duration flash of light, all PSII reaction centres will close (Maxwell and Johnson, 2000).

Table 2.2: Chlorophyll fluorescence parameters used in studies of PSII performance (Baker, 2008).

Parameter	Definition	Physiological relevance
F F’	Fluorescence emission from dark- or light-adapted, respectively	Information on photosynthetic performance.
F_o F_o’	Minimal fluorescence from dark- and light-adapted leaf, respectively	Fluorescence level when Q_A is maximally oxidized (PSII centres is open)
F_m F_m’	Maximal fluorescence from dark- and light-adapted leaf, respectively	Fluorescence level when Q_A is maximally reduced (PSII centers closed)
F_v F_v’	Variable fluorescence from dark- and light-adapted leaf, respectively	PSII performing photochemistry (Q_A reduction)
F_v/F_m	Maximum quantum efficiency of PSII photochemistry	Maximum efficiency at which light absorbed by PSII during Q_A reduction
NPQ	Nonphotochemical quenching	Estimates the non-photochemical quenching from F_m to F_m’.Meaning the heat lost from PSII

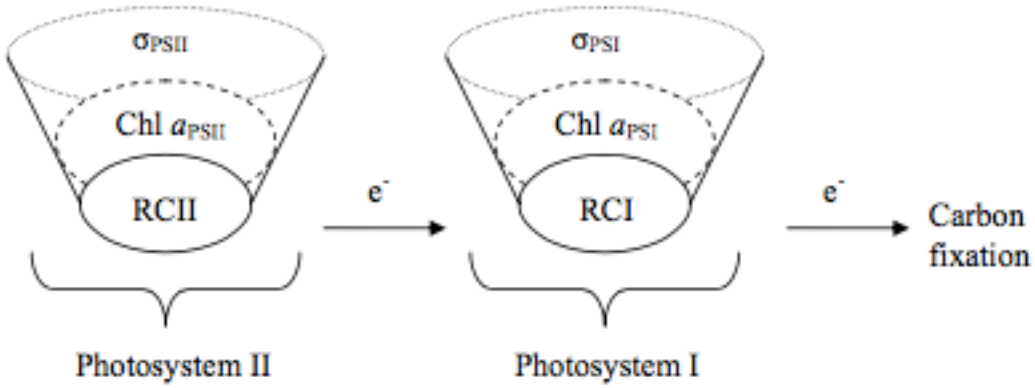


Figure 2.1: A schematic of photosystem II and I within the electron transport chain (Hennige, 2009).

Table 2.3: The increasing values of maximum quantum yield with depth from the surface water including summer and winter values. For the pooled data, these differences indicate that light and effect of nutrients can affect the quantum yields of photosynthesis (Dubinsky, 2013).

Locality	Depth	Maximum quantum yield	Reference
Sargasso Sea, Mauritania	Surface Surface	0.003 0.012	Morel (1978)
Pacific Ocean, South of Japan	Surface 10-20m 70m	0.004-0.01 0.01-0.026 0.026-0.075	Kishinoet et al. (1985)
Gulf of Eilat, Israel. Winter. Gulf of Eilat, Israel, summer.	Surface 80m Surface 80m	0.000025 0.110 0.000870 0.0266	Iluz (2008)
Lake Kinneret, Israel. Winter Lake Kinneret, Israel. Summer	Surface 3m Surface 5-7m	0.0025 0.043 0.0126 0.06	Dubinsky and Berman (1981)
Zooxanthellae *in hospice*		0.001 0.125	Dubinsky et al. (1984)

Dubinsky (2013) showed that maximum quantum yield cannot exceed 0.125 because the system would need four electrons for the evolution of one molecule of oxygen from water. Different regions, different depths, and different levels of lights on corals, showed different values of maximum quantum yield (Dubinsky, 2013). Values of maximum quantum yield can increase with depth from the surface to the deepest, as shown in Table 2.3. Morel (1978) recorded values at the surface of Sargasso Sea (nutrient-replete conditions) and the highly productive Mauritanian upwelling zone. Kishino et al. (1985) had found values increase at depth 70m (the deepest samples) in south of Japan. In Lake Kinneret, studies observed increase from surface with values 0.025 to 0.043 at 3 m depth (Iluz and Dubinsky, 2013).

Dubinsky (2013) also mentioned that the maximum quantum yield is measured during light-limited photosynthesis, where it is diagnosed by a linear relationship between photosynthesis and photosynthetic photons density. The response of photosynthesis is linear at low levels of radiation and saturates at high levels (Sharp et al., 1984). Different types of fluorometer give different methods and techniques to measure maximum quantum yield. The submersible pulse amplitude modulator (PAM) or Diving-PAM (Walz, Germany), uses saturating light pulse (0.8s, >2000μmol quanta m^{-2} s^{-1}) to measure its effective quantum yield readings and the change in fluorescence ($\Delta F = F_m - F_o$) is then used to calculate the effective quantum yield for under-natural sunlight specimens (Adzis et al., 2009). Cosgrove and Borowitzka (2010) recorded maximum theoretical values for F_v/F_m of ~0.65 for a single turnover saturation pulse and ~0.83 for multiple turnover pulses. They also mentioned that F_v/F_m is a variable based on a few factors such as between specimen taxa based on their pigment composition and cell structure, light, nutrient status and temperature.

Table 2.4 below shows the studies undertaken by researchers for effective quantum yield and maximum quantum yield of marine organisms in Malaysia. Mostly of the studies were recorded as effective quantum yield. Corals and anemone had found to have values of effective quantum yield between 0.6 to 0.7.

Table 2.4: Effective and maximum quantum yield of marine organisms in Malaysia

Locations	**Organisms**	**Effective quantum yield (F_v'/F_m')**	**Maximum quantum yield (F_v/F_m)**	**References**
Pulau Tioman, Pahang	Corals *Acropora formosa* and coral *Pocillophora damicornis*	0.65 0.7		Adzis et al. (2012)
Pulau Tioman, Pahang	Sea anemone, *Heteractis magnifica*	0.74±0.08		Khoo and Mazlan (2013)
Pulau Tioman, Pahang	Corals *Pocillophora damicornis*	0.70±0.05 0.72±0.03	0.70±0.20	Adzis et al. (2009)
Pulau Renggis Pulau Pangkor	Corals *Acropora formosa*	0.64 ± 0.096 0.67 ± 0.096 0.69 ± 0.135 0.72 ± 0.094		Xin et al. (2013)
Shore coastal Penang	Diatom genera *Cocconeis*, *Fragilaria*, *Paralia* and *Pleurosigma*		0.325 + 0.129	McMinn et al. (2005)

2.3 Pulse Amplitude Modulated (PAM) Fluorometer

Pulse amplitude modulation, or PAM, fluorometry operates on a basic signal modulation in which light is delivered in a series of signal pulses (Melbourne-Thomas, 2014). Biologists usually use PAM fluorometry to assess the photosynthetic state through comparison of photosynthetic rates based on fluorescence (Melbourne-Thomas, 2014).

Known to be a broader indicator of how plants respond to environmental change, PAM fluorometry is also easy to use and a non-invasive instrument (Murchie and Lawson, 2013). A user has to consider the type of measurement platform, instrument setting, timing of a period of dark adaptation and the conditions under which the specimens are measured (Murchie and Lawson, 2013). It is designed specifically to measure chlorophyll fluorescence yield at a high degree of sensitivity (Melbourne-Thomas, 2014).

PAM fluorometry has been widely used to analyse photosynthetic performance for green plants (Klughammer and Schreiber, 2008) and most kinds of photo-oxygenic algae (Ritchie and Bunthawin, 2010). PAM fluorometers have been developed to enable measurements of single cells through to the two-dimensional imaging of photosynthetic surfaces (Hill, 2008). PAM fluorometers are found to be suitable to estimate photosynthetic efficiencies of coral photosymbionts in vivo (Beer et al., 1998). Hill (2008) recorded that many versions of PAM fluorometers have been used in coral-related research, such as Microscopy PAM, Water-PAM, Microfibre-PAM, Mini-PAM, Diving-PAM and Imaging-PAM. PAM fluorometry can measure the light photons that are emitted as far-red fluorescence (~750 nm) from a flash of blue (~430nm) or red (~680nm) light (Ritchie and Bunthawin, 2010). All PAM fluorometers produce

a series of modulated pulses of measured light microseconds in duration) which induce the fluorescence excitation (Melbourne-Thomas, 2011).

Microscopy, Microfiber and Water –PAM differ from each other based on the colour type of LED mounted on the instrument, each of which can measure different types of cells. The MICROSCOPY-PAM, with a blue LED mounted as pulsed light source in an epifluorescence microscope with the photomultiplier being mounted on top of the microscope ocular, can be used for measurements at the level of single cells and chloroplasts (Walz, 1999). The MICROFIBER-PAM, with a blue, green, yellow or red LED as pulsed light source and the photomultiplier connected to a microfiber coupler, is used for small spot measurements down to single cells in different tissue layers (Walz, 1999). The WATER-PAM, with an array of measuring and actinic LEDs illuminating a water sample (e.g. from lakes, rivers, oceans) and special optics collecting the fluorescence on the photomultiplier, is used for the assessment of low levels of natural phytoplankton (Walz, 1999). The Mini-PAM has a Leaf-Clip Holder 2030-B for specificall gaining measurements from whole leaves (Walz, 2000). Imaging-PAM fluorometers are more recent versions of MAXI-, MINI-, MICRO- and MICROSCOPY with Multi Control Unit IMAG-CM, allowing large scale samples and microscopically small samples to be fluorescence imaged (Walz, 2005).

The WATER-PAM chlorophyll fluorometer was originally designed to measure plankton or leaf samples *ex-situ*. It is specialized for studying the unicellular algae, cyanobacteria, isolated chloroplasts and protoplasts (Walz, 1999). The instrument has 3 kinds of emitter-detector unit: cuvette version, fiber version and flow-through. For the specific purpose of this study to assess the photosynthetic activity of phytoplankton in surface water, the fiber version was used. The PAM-CONTROL Universal Control is similar to a MINI-PAM Chlorophyll Fluorometer, which

is a highly portable device for *ex-situ* studies (Waltz, 1999). The FIBER version probe is an optical fiber, to examine the photosynthetic layers on submerged surfaces (Walz, 1999). For this study, the Mini Quantum Sensor US-MQS/WB was used together with the WATER-PAM FIBER version to achieve the objectives. For chlorophyll measurement, WATER-PAM chlorophyll fluorometer is limited to the basis of the Fo level of a dark acclimated sample, which is the maximum quantum yield measurements (Walz, 1999). Portable fluorometers can measure photosynthetic performance in water, but unlike the Diving-PAM, Water-PAM's portable flurometer cannot be taken underwater and therefore have limited applications. A longer probe was used during this study to conduct measurement on aquarium corals underwater being accurately positioned on the surface of the coral nubbins to get the best fluorescence values. If the optic fibre is positioned on the shaded parts of the corals, it will affect the results by giving low values compared to well illuminated parts. The sensitive probe must be handled with care during the measurement, being straight, steady and directly on the samples to get consistent measurements. Before starting the experiments, various measurements of fluorescence yield around the coral nubbins was done to get the best results. ThePAM fluorometer has mostly been used for measuring stresses (Beer and Axelsson, 2004) and isthe only tool identified to measure photosynthetic of marine macroalgae under *in situ* conditions (Beet et al., 2010).

The key measurement by a PAM fluorometer is the proportion of photons of incident light that drives the photochemistry of photosynthesis, which is known as **effective quantum yield of photosynthesis, (*Y*)** (Ritchie and Bunthawin, 2010). PAM fluorometers are made for practical applications in ecophysiology with credibility of portability, tolerance of unfiltered sun light and diagnostic techniques for stress induced changes (Schreiber et al., 2000). Standard PAM fluorometery is non-invasive, using PIN-photodiodes to react as fluorescent detectors (Schreiber, 1998). The fluorescence measurements can be made rapidly, and because of the

portable nature of many fluorimeters, *in situ* (Baker, 2005). Nowadays, chlorophyll *a* fluorescence measurements have been used commonly to examine photosynthetic performance and stress in algae and plants, either through physiological or ecophysiological studies (Baker, 2008). This measurement is based upon the fluorescence properties of PSII, and that is essential for understanding the processes involved in the light reactions of PSII (Consalvey et al., 2005).

The fluorometer utilises light emitting diodes to produce a series of saturating pulses as well as actinic light, to determine the maximum and effective quantum yield (Fv/Fm); the parameters for PSII photochemical efficiency.

A dark-adapted value of F_v/F_m is used as a sensitive indicator of plant photosynthetic performance (Maxwell and Johnsson, 2000). The ratio of variable to maximal fluorescence in a darkened sample is correlated to the quantum yield of photosynthesis and is a convenient measure of the maximum potential quantum yield (Jones et al., 1999). To estimate the F_v/F_m, the value must need F_o and F_m (Maxwell and Johnson, 2000). Background fluorescence, F_o and maximal fluorescence value, F_m can be attained by the fluorometer by darkening the sample, and applies far-red illumination of wavelength >680nm for a few seconds (Maxwell and Johnson, 2000). F_m is where the photochemical efficiency is at maximum and the heat dissipation (excess energy of photosynthesis) is at a minimum value (Maxwell and Johnson, 2000). It is used as a quantitative measure of photoinactivation during coral bleaching (NOAA). The effects of coral bleaching on fluorescence were recorded to be conducted in hours to days' periods and under extreme bleaching conditions but not long-term changes (Rodrigues et al., 2008). But, if there is variability in fluorescence yield of many coral species, this can help research to predict long-term coral resilience of bleaching situations (Rodrigues et al., 2008). In a study, *Porites compressa* and *Montipora capitata* showed a decreased of F_v/F_m with 84% of control values,

after 2 weeks' exposure to temperature stress of 30.1±0.05°C. For the recovery stage, both coral species showed different responses in the photochemical efficiency of zooxanthellae in the normal quantum yield of PSII (Rodrigues et al., 2008). For *Pocillopora damicornis* in a temperature and solar radiation stress experiment, the DIVING-PAM (Heinz-Walz, Effeltrich, Germany) had recorded a reduced maximum quantum yield (below 0.4) from an average quantum yield of 0.6 to 0.8 during medium and high solar radiation treatments (Barron et al., 2010). During bleaching in Florida Keys, PAM fluorometer recorded damages of PSII of *Montastrea faveolata* and *Montastrea franski* during elevated seawater temperature with a significant declination of photosynthetic efficiency by depth (Warner et al., 1999). In a long-term study of elevated temperature stress, photosynthetic efficiency (F_v/F_m ratio) of *M. annularis* and *Agaricia agaricites* were found to be significantly decreased at high temperature of 32°C and *Agaricia lamarki* at 34°C (Warner et al., 1996).

2.4 Variables Fluorescence

Variable fluoroescene may be affected by photoacclimation, nutrient status and pollutants (Iluz and Dubinsk, 2013). When photoinhibition happens, it also gives low quantum yields. High irradiances can cause temporary and irreversible damages towards the photosynthetic apparatus (Iluz and Dubinsk, 2013). Different light levels measurement of maximum quantum yields (Dubinsky et al., 1986). Nutrient limitations (nitrogen, iron and phosphorus) can lower quantum yield (Iluz and Dubinsky, 2013). Variable fluorescence may also happen during light and dark reactions of photosynthesis experiments, where there is fluctuating levels of CO_2 (Maxwell and Johnson, 2000). Specialized leaf cuvettes or chambers can be used to fix this problem and the fibre optic has to be put at 45-60° angle (Maxwell and Johnson, 2000).

2.5 Assessing Coral bleaching using a PAM fluorometer

To monitor bleaching by using intrusive techniques involves removing coral tissue to extract the symbiotic dinoflagellates to measure the algal density, pigment content and tissue biomass processes (Fitt et al., 2001). Quantifying enzymes of chloroplast membranes is also one of the intrusive techniques, to identify coral bleaching from oxidative stress (Fitt et al., 2001). PAM fluorometry is an efficient non-intrusive alternative method of collecting data *in situ* and in laboratory conditions for monitoring the physiology of symbiotic dinoflagellates during coral bleaching conditions. An optimum photosynthesis efficiency, F_v/F_m value is 0.5-0.65, and depends on species and the water depth (Fitt et al., 2001). For a dark-adapted sample of *M. digitata* and *S. pistillata,* their F_v/F_m were valued between 0.65 to 0.7, which is typical for marine algae (Jones and Hoegh-Guldberg, 2001). If there is a decline in F_v/F_m values, it not only shows the algal stress or dysfunction, but also can reflect the seasonal or daily variation of a normal photosynthetic efficiency of a coral (Fitt et al., 2001). In coral species of *M. digitata* and *S. pistillata*, F_v/F_m showed a decrease during morning to noon, but increased during afternoons, demonstrating that there is a diurnal change in the quantum yields (Jones and Hoegh-Guldberg, 2001). The reduced fluorescence is caused by inhibition of the photo-oxidizing side of PSII during the heat exposure (Warner et al., 1996). When a chlorophyll molecule is in high radiation, there will be changes in the PSII reaction centre and a decline in the D1 reaction centre protein (Warner et al., 1999). The quantum yield of PSII that is measured by a PAM fluorometer shows the status of the photosynthetic activity and can be proportional to oxygen production or carbon dioxide uptake (Warner et al., 1999; Jones and Guldberg, 1999). Photoinhibition resulted from the energy excitation from PSII, damages on PSII or both (Jones and Hoegh-Guldberg, 2001). Coral bleaching has been related to thermal stress which lead to photoinbition in zooxanthellae (Bhagooli and Hidaka, 2004). The heat stress can cause chlorophyll fluorescence quenching,

where **photochemical (qP)** and **non-photochemical (qN)** components in the photosynthesis process are separated (Jones et al., 1998). When thermal and light stress occurs, there will be an increase of the reactive oxygen species (ROS) by the zooxanthellae, which damage the D1 protein in PSII (Hennige et al., 2001). In Jones and Hoegh-Guldberg's (2001) study, bleaching *S. pistillata* showed bleaching signs after being exposed to high light alone, indicative of photoinhibition of algal photosynthesis. Fluorescence analysis can detect different photosynthetic efficiency at specific sites in a coral colony (Warner et al., 1999). For *Montipora annularis* species, during a heat stress of 31.5°C, its fluorescent maximum quantum yield at the uppermost location of the colony were lower than the sides of the colony (Warner et al., 1999), probably because there are fewer zooxanthellae in the growing regions

PAM fluorometry has become the efficient method of collecting data in laboratory conditions and also *in situ* for the information on photosynthetic state of symbiotic algae (Fitt et al., 2001). Warner (2005) mentioned that F_v/F_m is the most commonly used parameter for assessing stressed corals, because it is easy to use and understand.

In laboratory studies, bleaching can occur due to synergistic factors such as high temperatures, high irradiance, high temperatures and nitrates or phosphates (Zhu et al., 2004; Wiedenmann et al., 2013); or t chemical cyanide poison (Jones and Hoegh-Guldberg, 1999; Douglas, 2003). By studying the chlorophyll fluorescence of coral species with short-term experiments with different temperature-irradiance and temperature-nitrate may provide information on the susceptibility of species towards bleaching conditions. *S. pistillata* showed a decrease of F_v/F_m at 26°C combined with high irradiance but *Platygyra ryukyuensis* had shown its F_v/F_m reductions started at 32°C and above, with all light exposures (520 and 110 μmol quanta m^{-2} s^{-1}) (Bhagooli and Hidaka, 2004). Hennige et al. (2011) found the different results of chlorophyll fluorescence measurement

of bleaching tolerated coral, *Porites asteroids* and bleaching sensitive coral, *M. faveolata* indicative of their thermal tolerance capabilities.

The relationship between fluorescence and photosynthesis is based on energy conversion theory and biophysical models (Parkhill et al., 2001). The portable PAM fluorometer) was used to examine the photosynthetic efficiency of endolithic algae inhabiting the skeletons of the corals under elevated treatments. The photosynthetic quantum yield of a plant or alga can most basically be defined as the amount of photosynthesis at a certain irradiance (P_I) per quanta of light (photons) absorbed (l_a) by the photosynthetic pigments (Saroussi and Beer, 2007). The PAM was calibrated so that the settings (gain = 7, damp = 2, measuring intensity = 7) produced initial fluorescence (F_o) of ~300-500 when a weak pulsed blue light was applied (3µs pulses of a light emitting diode, LED, peak emission at 650 nm) to dark-adapted corals of both species. A saturating pulse of bright white light (8000 µmol quanta $m^{-2}s^{-1}$ PAR, 800 ms pulse width) was then applied to give the maximal fluorescence value (F_m). The ratio of variable fluorescence (F_v, where $F_v = F_m - F_o$) to F_m in a dark-adapted sample (dark-adapted F_v/F_m) was used as a convenient measure of the maximum potential quantum yield (Jones and Hoegh-Guldberg, 2001). Most research uses the method of putting the corals in dark-adaptation for 20 min before F_v/F_m was determined for an optimum maximum quantum yield (F_v/F_m). It is recommended to set the distance (5mm) (Ferrier-Pages et al., 2007) between the sample and the optic fibre of the PAM as constant for each sample so that accurate measurement of chlorophyll fluorescence can be achieved (Bhagooli and Hidaka, 2004).

2.6 Water-PAM fluorometer

The Water-PAM fluorometer measures parameters such as chlorophyll content and photosynthetic activity of aquatic species, allowing for the optical probe to be immersed. Thus, this study used the instrument to achieve the objectives. Basically, PAM units consist of three major components; the PAM-CONTROL universal control unit, fluorescence emitter-detector unit and WinControl Windows software for operation with a computer. The PAM-CONTROL universal control unit is the largest component of the instrument, and runs the PAM electronically. The fluoresence emitter-detector unit contains an array of LED diodes with red (650-660nm) for fluorescence excitation, actinic illumination and saturation pulses. The PAM can be controlled by a computer by using the WinControl Windows software.

To collect data for the Water-PAM fluorometer, the samples need to be dark-adapted before placing the emitter-detector unit within 5 mm of the sample to get maximum quantum yield measurement. Data is accepted in the control unit as saturation pulses or known as rapid light curves (RLCs). The PAM unit is able to store around 5000 data lines. The logged data can be transferred to computer via WinControl software as a 'Report file'. The fluorescence emitter unit that was used in this study is the Mini Quantum Sensor US-MQS/QB. To get started, the PAM-CONTROL unit is connected to the Water-PAM emitter detector unit and is set at PM-GAIN 15 and OUT-GAIN 2 which are the effective standard settings. After pressing the START key, the saturation pulse is triggered and yield measurement can be measured. The display of the PAM-control is shown in Fig. 2.2.

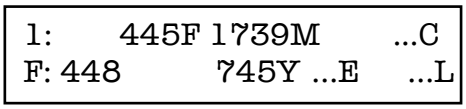


Figure 2.2: Displayed parameters on PAM-CONTROL screen after START key is pressed.

1:	Number count for each time the START key is pressed
445F	Fluorescence yield (F) measured briefly before the last saturating light pulse triggered by START: 445 is the example based of the display screen above.
1739M	Maximal fluorescence yield (= Fm or Fm')
…C	Temperature in degree Celsius
F: 448	Momentary fluorescence yield displaying small fluctuations
745Y	Yield-parameter
…E	Relative of electron transport (ETR)
…L	Light intensity in terms of PAR

In this study, the yield parameter by the saturation pulse method was recorded as F_v/F_m, maximum quantum yield, for the assessment of the photosynthetic performance of the sample.

References

AFP, 2010. Malaysia closes top diving sites due to coral bleaching. In: Sydney Morning Herald [online]: http://www.smh.com.au/travel/travel-news/malaysia-closes-top-diving-sites-due-to-coral-bleaching- 20100723-10o22.html.

Adzis, K. A. A., Amri, A. Y., Hyde, J., Maidin, N., Repin, I. M., Ling, A. S., & Mohamed, C. A. R. (2012). In situ measurement of photosynthetic capacity in Scleractinian corals (*Acropora formosa* and *Pocillopora damicornis)* during the 2010 massive coral reef bleaching event in Pulau Tioman, Malaysia. *Journal of Science and Technology in the Tropics* **8**: 85-96

Adzis, K. A. A., Amri, A. Y., Oliver, J., & Mohamed, C. A. R. (2009). Effective and maximum quantum yield of the lace coral *Pocillopora damicornis* (Anthozoa: Scleractinia: *Pocilloporidae*) in Pulau Tioman, Malaysia. *Journal of Science and Technology in the Tropics* **5(1):** 13-17.

Atkinson M.J., Carlson B., Crow G.L. 1995. Coral growth in high nutrient, low-pH seawater: a case study of corals cultured at the Waikiki Aquarium, Honolulu, Hawaii. *Coral Reefs* **14:** 215-223.

Banin, E., Israely, T., Kushmaro, A., Loya, Y., Orr, E., & Rosenberg, E. 2000. Penetration of the coral-bleaching bacterium *Vibrio shiloi* into *Oculina patagonica*. *Applied and Environmental Microbiology*, **66 (7):** 3031-3036.

Baker A. 2003. Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics,* **34**: 661-689.

Baker N.R. 2008. Chlorophyll fluorescence: A probe of photosynthesis in vivo. *The Annual Review of Plant Biology* **59:** 89-113.

Barron M.G., McGill C.J., Courtney L.A., and Marcovich D.T. 2010. Experimental Bleaching of a Reef-Building Coral Using a Simplified Recirculating Laboratory Exposure System. *Journal of Marine Biology*, pp. 8.

Beer S. and Axelsson L. 2004. Limitations in the use of PAM fluorometry for measuring photosynthetic rates of macroalgae at high irradiances. *European Journal of Phycology* **39 (1):** 1-7.

Birkeland, C., 1997. Introduction. pp. 1 –12. In: Birkeland, C. (Ed.), Life and Death of Coral Reefs. Chapman and Hall, New York. 536 pp.

Brown B.E. and Bythell J.C. 2005. Perspective on Mucus Secretion in Reef Corals. *Marine Ecology Progress Series* **296:** 291-309.

Burke L., Selig E. and Spalding M. 2002. Reefs at risk in Southeast Asia. World Resources Institute. United States of America.

Bongaerts P, Riginos C, Ridgway T, Sampayo EM, van Oppen MJH, Englebert N, et al. 2010. Genetic Divergence across Habitats in the Widespread Coral *Seriatopora hystrix* and Its Associated Symbiodinium. *PLoS ONE* **5(5):** e10871. doi: 10.1371/journal.pone.0010871

Björkman, O., & Demmig, B. (1987). Photon yield of O 2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta,* **170(4)**: 489-504. Retrieved from http://www.jstor.org/stable/23379014.

Carlson B.A. 1987. Aquarium Systems for Living Corals. International Zoo Yearbook **26:** 1-9.

Császár N.B.M., Ralph P.J., Frankham R., Berkelmans R., J.H. van Oppen. 2010. Estimating the potential for adaptation of corals to climate warming. PLos ONE **5**(3): e9751.

Cros A., Ahamad Fatan N., White A., Teoh S.J., Tan S., Handayani C., Huang C., Peterson N., Li R.V., Siry H.Y., Fitriana R., Gove J., Acoba T., Knight M., Acosta R., Andrew N. And Beare D. 2014. The Coral Triangle atlas: An integrated online spatial database system for improving coral reef management. PLoS ONE **9**.

Dong. Z.-J., Huang H., Huang L.-M. and Li Y.-C. 2009. Diversity of symbiotic algae of the genus *Symbiodinium* in scleractinian corals of the Xisha Islands in the South China Sea. *Journal of Systematics and Evolution*, **47**: 321–326.

Dordrecht. 1998. Chlorophyll Fluorescence: New Instruments for Special Applications In: Garab G. (ed) Photosynthesis: Mechanisms and Effects. Vol. V, pp. 4253-4258. Kluwer Academic Publishers, Würzburg, Germany.

Ebeid M.L., Hassan M.H. and Geneid Y.A. 2009. Response to increased sediment load by three coral species from the Gulf of Suez (Red Sea). *Journal of Fisheries and Aquatic Science* **4(5):** pp. 238-245.

Fenner D. 2001. Malaysian Hard Corals. *Department of Marine Parks Malaysia, Putrajaya.*

Ferrier-Pagès C., Gattuso J.-P. And Jaubert J. 1999. Effect of small variations in salinity on the rates of photosynthesis and respiration of the zooxanthellae coral *Stylophora pistillata*. *Marine Ecology Progress series* **181:** 309-314.

Fernandez-Jaramillo, Arturo A.; Duarte-Galvan, Carlos; Contreras-Medina, Luis M.; Torres-Pacheco, Irineo; Romero-Troncoso, Rene de J.; Guevara-Gonzalez, Ramon G.; Millan-Almaraz, Jesus R. 2012. "Instrumentation in Developing Chlorophyll Fluorescence Biosensing: A Review." *Sensors* **12 (**9): 11853-11869.

Ferrier-Pagès C., Schoelzke V., Jaubert J., Muscatine L. And Hoegh-Guldberg O. 2001. Response of a scleractinian coral, *Stylophora pistillata*, to iron and nitrate enrichment. *Journal of Experimental Marine Biology and Ecology* **259:** 249-261.

Fitt W.K., Gates R.D., Hoegh-Guldberg O., Bythell J.C., Jatkar A., Grottoli A.G., Gomez M., Fisher P., Lajuenesse T.C., Pantos O., Iglesias-Prieto R., Franklin D.J., Rodrigues L.J., Torregiani J.M., van Woesik R. and Lesser M.P. 2009. Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata*, to short-term thermal stress: The host does matter in determining the tolerance of corals to bleaching. *Journal of Experimental Marine Biology and Ecology* **373:** 102-110.

Fitt, W.K., Brown, B.E., Warner, M.E. and Dunne, R.P., 2001. Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* **20** (1): 51-65.

Franklin L.A., Seaton G.G.R., Lovelock C.E. & Larkum A.W.D. (1996) Photoinhibition of photosynthesis on a coral reef. *Plant, Cell and Environment* **19**: 825–836.

Fitt W.K. and Warner M.E. (1995) Bleaching patterns of four species of Caribbean reef corals. Biology Bulletin **189:**298–307.

Franklin D.J., Hoegh-Guldberg O., Jones R.J. and Berges J.A. 2004. Cell death and degeneration in the symbiotic dinoflagellates of the coral *Stylophora pistillata* during bleaching. *Marine Ecology Progress Series* **272:** 117-130.

Franklin D.J., Molina Cedrés C.M. and Hoegh-Guldberg O. 2006. Increased mortality and photoinhibition in the symbiotic dinoflagellates of the Indo-Pacific coral *Stylophora pistillata* (Esper) after summer bleaching. *Marine Biology* **149:** 633-642.

Gorbunov, M.Y. and Falkowski, P.G., 2004, August. Fluorescence induction and relaxation (FIRe) technique and instrumentation for monitoring photosynthetic processes and primary production in aquatic ecosystems. In *Photosynthesis: Fundamental Aspects to Global Perspectives"-Proc. 13th International Congress of Photosynthesis, Montreal, Aug*. pp. 1029-1031.

Grover R., Maguer J-F., Allemand D. and Ferrier-Pagès C. 2003. Nitrate uptake in the Scleractinian coral *Stylophora pistillata*. *Limnology and Oceanography* **48** (6): 2266-2274.

Harborne A., Fenner D., Barnes A., Beger M., Harding S. and Roxburgh T. 2000. Status report on the coral reefs of the east coast of Peninsula Malaysia. *Report by Coral Cay Conservation Ltd.*

Hennige, S.J., 2009. *Photoacclimation and adaptation of hermatypic coral species across environmental gradients* (Doctoral dissertation, The University of Essex).

Hoegh-Gulberg O. and Smith G.J. 1989. The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. *Journal of Experimental Marine Biology and Ecology* **129:** 279-303.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Marine and freshwater research. **50** (8): 839-866.

Hoeksema B., Rogers, A. and Quibilan, M. 2008. *Stylophora pistillata.* In IUCN 2013. IUCN Red List of Threatened Species.

Hongo C. And Yamano H. 2013. Species-specific responses of corals to bleaching events on anthropogenically turbid reefs on Okinawa Island, Japan, over 15-year period (1995-2009). *PLoS ONE* **8**(4): 360952.

Hoogenboom M.O., Campbell D.A., Beraud E., DeZeeuw K., Ferrier-Pagès C. 2012. Effects of light, food availability and temperature stress on the function of photosystem II and photosytem II of coral symbions. PLoS ONE **7**(1).

Hyde J., Chelliah A.J., Chen S.Y. 2013. Reef Rehabilitation Experiments: A Review of Results & Lessons Learned. Reef Check Malaysia.

Hyde J., Yee C.S. and Chelliah A. 2013. Five years of Reef Check monitoring data for Tioman, Perhentian and Redang Island. *Malaysian Journal of Science* **32**: 117-126.

Iluz D. and Dubinsky Z. 2013. Quantum yields in aquatic photosynthesis. Chapter in book *Photosynthesis.*

Jimenez Isabel M., Larkum Anthony W.D., Ralph Peter J., KÜhl M. 2012. Thermal effects of tissue optics in symbiont-bearing reef-building corals. *Limnology and Oceanography*, **57**.

Jones, R. J. and Hoegh-Guldberg, O. 2001. Diurnal changes in the photochemical efficiency of the symbiotic dinoflagellates (Dinophyceae) of corals: photoprotection, photoinactivation and the relationship to coral bleaching. Plant, Cell & Environment, **24**: 89–99.

Jones R.J., Kildea T. and Hoegh-guldberg O. 2004. PAM chlorophyll fluorometry: a new *in situ* technique for stress assessment in scleractinian corals, used to examine the effects of cyanide from cyanide fishing.

Jonsson D. 2003. An inventory of coral reefs in Langkawi Archipelago, Malaysia – Assessment and impact study of sedimentation. *Undergraduate Thesis University of Sweden.*

Juneau P. And Popovic R. 2000. Evidence for the rapid phytotoxicity and environmental stress evaluation using PAM fluorometric method: importance and future application. *Ecotoxicology* **8**: 449-455. Kluwer Academic Publishers.

Kemp D.W., Oakley C.A., Thornhill D.J., Newcomb L.A., Schmidt G.W. and Fitt W.K. 2011. Catastrophic mortality on inshore coral reefs of the Florida Keys due to severe low-temperature stress. Global Change Biology 17(11): 3468-3477.

Khoo M.L. and Mazlan G.A. 2013. Diurnal patterns of the effective quantum yield of the magnificent sea anemone *Heteractis magnifica* in Pulau Tioman, Malaysia. *Galaxea, Journal of Coral Reef Studies (Special Issue)*. pp. 133-137.

Klughammer, C., & Schreiber, U. (2008). Complementary PSII quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. PAM Application Notes, 1(2).

Koren S., Dubinsky Z. and Chomsky O. 2008. Induced bleaching of *Stylophora pistillata* by darkness stress and its subsequent recovery. *Proceedings of the 11th International Coral Reef Symposium, Florida, 7-11 July 2008.*

Krueger T., Hawkins T.D., Becker S., Pontasch S., Dove S., Hoegh-Guldberg O., Leggat W., Fisher P.L. and Davy S.K. 2015. Differential coral bleaching – contrasting the activity and response of enzymatic antioxidants in symbiotic partners under thermal stress. *Comparative Biochemistry and Physiology* **Part A 190:** 15-25.

LaJeunesse T.C., Loh W.K.W., Van Woesik R., Hoegh-Guldberg O, Schmidt G.W. and Fitt W.K. 2003. Low symbiont diversity in Southern Great Barrier Reef Corals, relative to those of the Carribean. *Limnology and Oceanograhy* **48**(5): 2046-2054.

Latypov Y.Y. 2007. Free-living Scleractinian Corals on Reefs of the Seychelles Islands. *Russian Journal of Marine Biology* **33**(4): 222-226.

Li S., Yu K.F, Shi Q., Chen T.R, Zhao M.X. and Zhao J.X. 2008. Interspecies and spatial diversity in the symbiotic zooxanthellae density in corals from northern South China Sea and its relationship to coral reef bleaching. *Chinese Science Bulletin* **53**(2): 295-303.

Liu, G., A. E. Strong, and W. Skirving (2003), Remote sensing of sea surface temperatures during 2002 Barrier Reef coral bleaching, Eos Trans. AGU, 84(15), 137–141, doi:10.1029/2003EO150001.

Loh W.K.W., Loi T., Carter D. and Hoegh-Guldberg O. 2001. Genetic variability of the symbiotic dinoflagellates from the wide-ranging coral species *Seriatopora hystrix* and *Acropora longicyathus* in the Indo-West Pacific. Marine Ecology Progress Series **222:** 97-107.

Maps of Malaysia (2017, February 27). Retrieved from reefgis.reefbase.org.

Marshall, P. and Schuttenberg, H. 2006. A Reef Manager's Guide to Coral Bleaching. Townsville, Australia, Great Barrier Reef Marine Park Authority.

Maxwell K. and Johnson G.N. 2000. Chlorophyll fluorescence – a practical guide. *Journal of Experimental Biology* **51 (345):** 659-668.

Mayfield A.B, Chan P.H., Putnam H.M., Chen C.S., and Fan T.Y. 2012. The effects of a variable temperature regime on the physiology of the reef-building coral *Seriatopra hystrix:* results from a laboratory-based reciprocal transplant. *The Journal of Experimental Biology* **215:** 4183-4195.

Mazlan A.G., Zaidi C.C., Wan-Lotfi W.M. and Othman B.H.R. 2005. On the current status of coastal marine biodiversity in Malaysia. Indian Journal of Marine Sciences **34 (1):** 76-87.

Misra, A.N., Misra, M. and Singh, R., 2012. *Chlorophyll fluorescence in plant biology*. INTECH Open Access Publisher.

Murchie E.H. and Lawson T. 2013. Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany*.

Muller-Parker, G. 1984. Photosynthesis-irradiance responses and photosynthetic periodicity in the sea anemone *Aiptasia pulchella* and its zooxanthellae. Marine Biology, 82(3), 225-232.

Muscatine, L., and Cernichiari, E. 1969. Assimilation of photosynthetic products of zooxanthellae by a reef coral. The Biological Bulletin, **137(3):** 506-523.

Pilcher, N. and Cabanban, A., 2000. The status of coral reefs in eastern Malaysia. Global Coral Reef Monitoring Network (GCRMN) Report. Australia Institute of Marine Science, Townsville.

Putnam H.M., Edmunds P.J. and Fan T.Y. 2008. Effect of temperature on the settlement choice and photophysiology of larvae from the reef coral *Stylophora pistillata*. *Biology Bulletin* **215:** 135-142.

Ralph, P.J., Gademann, R. and Larkum, A.W., 2001. Zooxanthellae expelled from bleached corals at 338C are photosynthetically competent. *Marine Ecology Progress Series* **220:** 163-168.

Ralph P.J., Schreiber U., Kuhl M. And Larkum A.W.D. 2005. Coral photobiology studied with a new imaging pulse amplitude modulated fluorometer. *Journal of Phycology*. **41 (2):** 335-342.

Ritchie, R. J., & Bunthawin, S. 2010. The use of pulse amplitude modulation (PAM) fluorometry to measure photosynthesis in a CAM orchid, *Dendrobium* spp. (D. cv. Viravuth Pink). *International journal of plant sciences*. **171(6):** 575-585.

Reef Check Malaysia. 2016. Status of coral reefs in Malaysia, 2015. In RCM Annual Survey Report. Reef Check Malaysia, Kuala Lumpur.

Reef Check Malaysia. 2016. Improving marine resource management in Malaysia, towards greater community participation. In RCM Advocacy Report 2016. Reef Check Malaysia, Kuala Lumpur, Malaysia.

Revsbech N. P. 1995. Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for 0^2, pH and light. *Marine ecology progress series*. **117:** 159-172.

Riegl B. And Velimirov B. 1991. How many damaged corals in Red Sea reef systems? A quantitative survey. *Hydrobiologia* **216/217:** 249-256.

Roth, M.S., 2014. The engine of the reef: photobiology of the coral–algal symbiosis. *Frontiers in microbiology* 5**.**

Saleh, M.S.M. and Hasan, N.N.N., 2014. Ecotourism and Coral Reef: Analysis of Coral Reef Conservation Awareness among Stakeholders in Perhentian Island, Malaysia. *American Journal of Tourism Management*, ***3*****(1A):** 7-12.

Salih, A., Larkum, A., Cox, G., Kühl, M., & Hoegh-Guldberg, O. (2000). Fluorescent pigments in corals are photoprotective. Nature, **408(6814):** 850-853.

Saroussi S. and Beer S. 2007. Alpha and quantum yield of aquatic plants derived from PAM fluorometry: Uses and misuses. *Aquatic Botany Elsevier* **86 (1):** 89-92.

Schreiber U. 2004. Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview. *In Chlorophyll a Fluorescence* (pp. 279-319). Springer Netherlands.

Schreiber, U., Bilger, W., Hormann, H., & Neubauer, C. 2000. 24 Chlorophyll fluorescence as a diagnostic tool: basics and some aspects of practical relevance. *Photosynthesis: a comprehensive treatise*, **320**.

Schreiber, U., Bilger, W., Klughammer, C., & Neubauer, C. 1988. Application of the PAM fluorometer in stress detection. In Applications of Chlorophyll Fluorescene in *Photosynthesis*

Research, Stress Physiology, Hydrobiology and Remote Sensing (pp. 151-155). Springer Netherlands.

Silverstein R.N., Cunning R. and Baker A.C. 2014. Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biology*.

Singsaas E.L., Ort D.R. and DeLucia E.H. 2001. Varitation in measured values of photosynthetic quantum yield in ecophysiological studies. *Oecologia* **128:** 15-23.

SOKI Wiki. 2014. Pulse Amplitude Modulated (PAM) Fluorometry - Methods – Confluence. SOKI, Antarctic Climate and Ecosystems Co-operative Research Centre. Accessed: Wednesday 17 Feb 2016.

Souter D. and Lindén O. 2005. Coral reef degradation in the Indian Ocean. CORDIO, Kalmar, Sweden.

Stimson J., Sakai K. and Sembali H. 2002. Interspecific comparison of the symbiotic relationship in corals with high and low rates of bleaching-induced mortality. *Coral reefs.* **21:** 409-421.

Thomas C.R. and Heron S.F. 2011. South-East Asia coral bleaching rapid response: Final Report. In National Research Flagships, Wealth from Ocens, CSIRO.

Titlyanov E., Bil' K., Fomina I., Titlyanova T., Leletkin V., Eden N., Malkin A. and Dubinsky Z. 2000. Effects of dissolved ammonium addition and host feeding with *Artemia salina* on photoacclimation of the hermatypic coral *Stylophora pistillata. Marine Biology* **137:** 463-472.

Titlyanov E.A., Titlyanova T.V., Yamazato K. and van Woesik R. 2001. Photo-acclimation dynamics of the coral *Stylophora pistillata* to low and extremely low light. *Journal of Experimental Marine Biology and Ecology* **263:** 211-225.

Toda, T., Okashita, T., Maekawa, T., Alfian, B. A. A. K., Rajuddin, M. K. M., Nakajima, R., & Terazaki, M. 2007. Community structures of coral reefs around Peninsular Malaysia. *Journal of Oceanography* **63(1):** 113-123.

Waheed Z. and Hoeksema B.W. 2013. A tale of two winds: species richness patterns of reef corals around the Semporna peninsula, Malaysia. *Marine Biodiversity* **43:** 37-51.

Waheed Z., van Mil H.G.J., Hussein M.A.S., Jumin R., Ahad B.G. and Hoeksema B.W. 2015. Coral reefs at the Northern most tip of Borneo: An assessment of Scleractinian species richness patterns and benthic reef assemblages. *PLoS ONE* **10 (12).**

Walz H. G. 1999. PAM-CONTROL Universal Control Unit: For ultrasensitive chlorophyll fluorescence measurements: Handbook of Operation. Revised May 2013. Germany.

Walz H.G. 2000. WinControl Windows Software for PAM Fluorometers: Users manual. Germany.

Walz H.G. 2005. Imaging-PAM M series: Chlorophyll Flurescence System. Germany.

Ward S., Harrison P., and Hoegh-Guldberg O. 2000. Coral bleaching reduces reproduction of scleractinian corals and increases susceptibility to future stress. *Proceedings 9th International Coral Reef Symposium*. Bali Indonesia.

Warner, M. E., Fitt, W. K. And Schmidt, G. W. 1996. The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae *in hospite* from four different species of reef coral: a novel approach. *Plant, Cell & Environment* **19:** 291–299.

Warner M.E., Fitt W.K. and Schmidt G.W. 1999. Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. *Proc. Natl. Acad. Sci*. **96:** 8007-8012.

Warner M.E. 2005. An overview of the interpretation and current use of chlorophyll fluorescence to understand coral bleaching. In: Hoegh Guldberg O (ed) Understanding the stress response of corals and *Symbiodinium* in a rapidly changing environment. Proceedings of the Bleaching Working Group Inaugural Workshop, Puerto Morelos, May 10–Jun 3, 2005, pp 8–9.

Waikiki Al-Naema N., Saeed S., Deb N. And Dupáont J. 2014. Pulse Amplitude Modulation Fluorometry technique and laboratory cultivation of Qatari Corals: Studying coral growth and photosynthetic efficiency. *Society of Petroleum Engineers*.

Winter G., Loya Y., Röttgers R. and Beer S. 2003. Photoinhibition in shallow-water colonies of the coral *Stylophora pistillata* as measured *in-situ*. *Limnology Oceanography* **48 (4):** 1388-1393.

Xin, L. H., Cheliah, A., Yee, C. S., Hyde, J., Cob, Z. C., & Adzis, K. A. A. Comparison of *Acropora formosa* coral growth in natural habitat condition between Tioman Island and Pangkor Island, Malaysia. *Journal of Science and Technology in the Tropics* **(2013) 9:** 31-45.

Yamashita H, Suzuki G, Kai S, Hayashibara T, Koike K (2014) Establishment of Coral–Algal Symbiosis Requires Attraction and Selection. *PLoS ONE* **9(5)**.

Yuen Y.S., Nakamura T., Yamazaki S.S. and Yamasaki H. (2008) Long-term effects of inorganic nitrogen enrichment on the reef-building corals *Stylophora pistillata* and *Acropora* spp. *Proceedings of the 11th International Coral Reef Symposium, Florida 7th-11th July 2008.*

Zack Z. 2012. Aquarium Corals: Applying PAM fluorometry for the advancement of coral aquaculture. Retrieved from http://www.advancedaquarist.com/2012/1/corals.

Chapter 3

Experimental Corals and Their Maintenance in Controlled Laboratory Conditions

In the present study, three Indo-Pacific coral species were used for the stress experiments of temperature-light and temperature-nitrate. *Stylophora pistillata*, *Montipora digitata* and *Seriatopora hystrix* came from a sustainable source and were maintained in laboratory conditions pre-experiments. This chapter explains the experimental coral species and how they are maintained in the laboratory.

3.1 Coral *Stylophora pistillata*

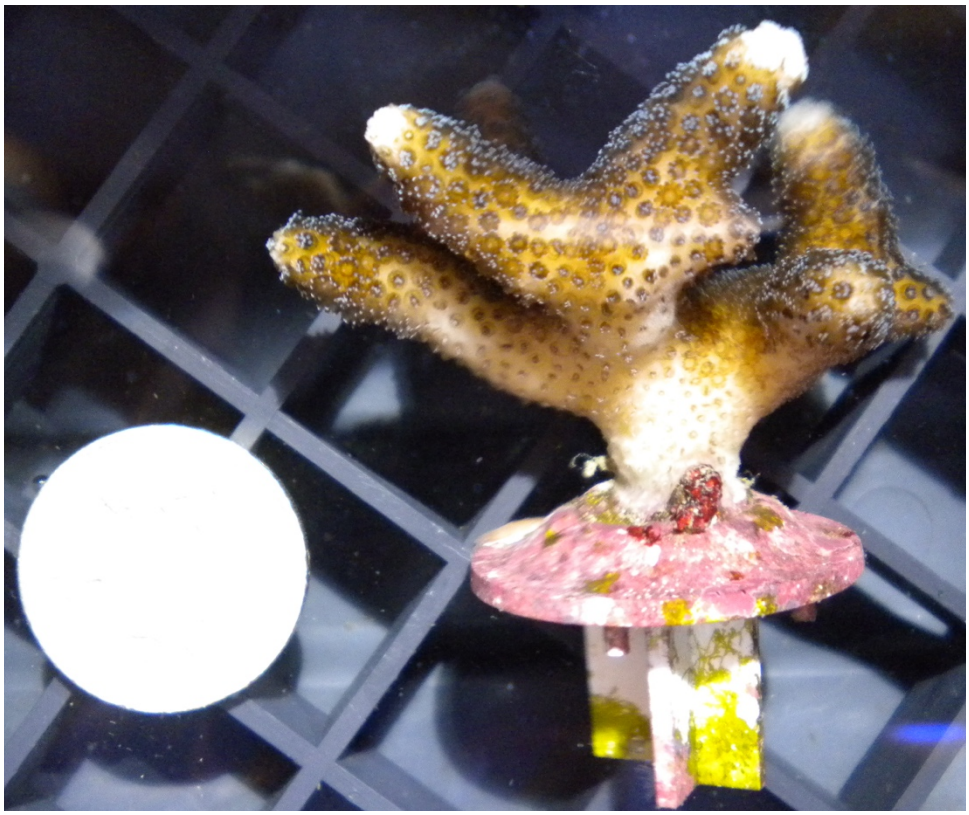


Figure 3.1: Coral *Stylophora pistillata*.

Figure 3.2: Distribution map of *S. pistillata* in the world (www.coralsoftheworld.org)

Stylophora pistillata (Esper, 1797) colonies can be found in the Indo-West Pacific, Red Sea and the Gulf of Aden, the southwest and northwest Indian Ocean, the Arabian/Iranian Gulf, the central Indian Ocean, the central Indo-Pacific, tropical Australia, southern Japan and the South China Sea, the oceanic West Pacific and the Central Pacific (Veron, 2017). Fig. 3.2 above shows the distribution of *S. pistillata* all around the world (brown label). In the South China Sea, they can be found at 3-11m seawater depth (Hoeksema et al., 2014). *S. pistillata* is highly variable in morphology and has a broad biogeographic range (Red Sea to western central Pacific) (Keshavmurthy et al., 2012) and is common in most shallow water reef areas as well as in deep water and lagoons (Ferrier-Pages et al., 1999). Its characteristics are branching with blunt-ended branches, thick and submassive (Veron, 2017). Colour ranges from uniform cream, pink, blue or green, depends on the light types they get (Veron, 2017). In Malaysia, *S. pistillata* were recorded abundantly in Pulau Tioman (Harbone et al., 2000). It has been the focus of coral research over the last four decades (Keshavmurthy et al., 2012) and has been used in most biological and physiological studies (Ferrier-Pages *et al*., 1999). Besides being identified as a thermo-tolerant species (Franklin et al., 2004), Koren et al. (2008) mentioned that this coral species recovered easily from experimental handling and breakage fragments, and so it is a

suitable sample for a laboratory-conditioned experiment. Riegl and Velimirov (1991) also mentioned a similar finding, even though *S. pistillata* is among species that suffer the most amount of damage in the Red Sea, but they are relatively fast growing and can tolerate repeated breakage.

3.1.1 *Stylophora pistillata* vs temperature and light

Hoegh-Guldberg and Smith (1989) found that in a reduced salinity or elevated solar irradiance condition, *S. pistillata* and *S. hystrix* displayed rapid expulsion of symbionts, but the zooxanthellae density returned to normal during recovery time in 19 days. *S. pistillata* was used to examine the effects of increased light, increased temperature and combinations of increase light and temperature by measuring photosynthetic efficiency; oxidative stress, cell viability and the algae morphology in studies of Franklin et al. (2004). Under short-term thermal stress, the coral's DNA was damaged more than in the coral *Porites cylindrical* after a stress of high temperature; 32°C for 5 days (Fitt et al., 2009). Franklin et al. (2006) also proved that high light and high temperature stress (31C (±1), 1,100 (±100) µmol m^{-2} s^{-1} may cause the mortality of the symbionts within the coral. *S. pistillata* bleached after dark stress, and recovered in 30 days after being returned to a light condition (Koren et al., 2008). *S. pistillata* was also found to adapt to a reduced light intensity, by an increase in both of chlorophyll concentration and zooxanthellae population density (Titlyanov et al., 2000). In the Red Sea, this scleractinian hard coral of the shallow area, showed a lower maximum quantum yield (F_v/F_m) than deeper growing ones (Winter et al., 2003) and a study by Putnam et al. (2008) has proved that temperature may affect settlement and physiology of the species coral larvae.

Table 3.1: Summary of *Stylophora pistillata* corals stress experiments of the latest references.

Coral species	Treatments	Results	Citations
S. pistillata and *S. hystrix*	reduced salinity or elevated solar irradiance condition	Rapid expulsion of zooxanthellae	Hoegh-Guldberg and Smith (1989)
S. pistillata	combinations of increase light and temperature	measuring photosynthetic efficiency; oxidative stress, cell viability and the algae morphology	Franklin et al., 2004
S. pistillata	High temperature; 32°C for 5 days	DNA was less more than *Porites cylindrical*	Fitt et al., 2009
S. pistillata	High temperature and high light; 31C (±1), 1,100 (±100) µmol m^{-2} s^{-1}	Coral symbionts' mortality	Franklin et al., 2006
S. pistillata	Darkness stress	Corals bleached and recovered in 30 days after returned to a light condition	Koren et al., 2008
S. pistillata	Reduced light intensity	Increased in chlorophyll concentration and zooxanthellae population density	Titlyanov et al., 2000
S. pistillata	Shallow and deeper water depth	Lower maximum quantum yield (F_v/F_m) than the deeper growing ones.	Winter et al., 2003

S. pistillata	23°C, 25°C (ambient), 29°C at light intensities of 150 mol photons m^{-2} s^{-1}.	Settlement and physiology of the species coral larvae were affected.	Putnam et al., 2008
S. pistillata	High temperature; 34°C	Reduction and less recovery in Fv/Fm of isolated zooxanthellae than normal temperature (28°C) under all light conditions.	Bhagooli and Hidaka, 2003
S. pistillata	Light: 0, 110, 520, 1015 µmol quanta $m^{-2}s^{-1}$ and temperatures; (26, 32 and 34ºC)	Paling and high mortality.	Bhagooli and Hidaka, 2004

3.1.2 *Stylophora pistillata* vs nutrients

S. pistillata was found to respond significantly (by bleaching) towards excessive inorganic nitrogen with the concentration of 0.001 mmol/L of ammonia or nitrate (Baohua *et al.* 2004). But in a similar study, the nitrate enrichment of 2 µM did not significantly change the zooxanthellae density or the photosynthesis rates for this specific coral species. Because the reef ecosystem is low in inorganic nutrients, Grover et al. (2003) studied the nitrate uptake by *S. pistillata* under high ambient nitrate concentration, high low nitrate concentrations, under different light intensities and with and without high ambient ammonium concentrations. The study found that the uptake of nitrate might be dependent on light intensities when corals are taking up both ammonium and nitrate. High nutrient, high-PCO_2 water of the Waikiki Aquarium

in Hawaii has supported growth of all the coral communities, including *S. pistillata* (Atkinson et al., 1995). Wiedemann et al. (2012) suggested that increased levels of dissolved inorganic nitrogen with limited phosphate concentrations can increase susceptibility to temperature and light-induced bleaching. According to Yuen et al. (2008), in a long term studies of nitrate enrichment effects on this specific coral species over 12 months, the survival rate, zooxanthellae density and maximum quantum yield (F_v/F_m) showed that *S. pistillata* can tolerate elevated nutrient levels better than fast-growing *Acropora* spp. *S. pistillata* is one of the species that were used in this study to know its susceptibility towards high and ambient levels of nitrate combined with high and ambient levels of temperatures.

3.2 Coral *Montipora digitata*

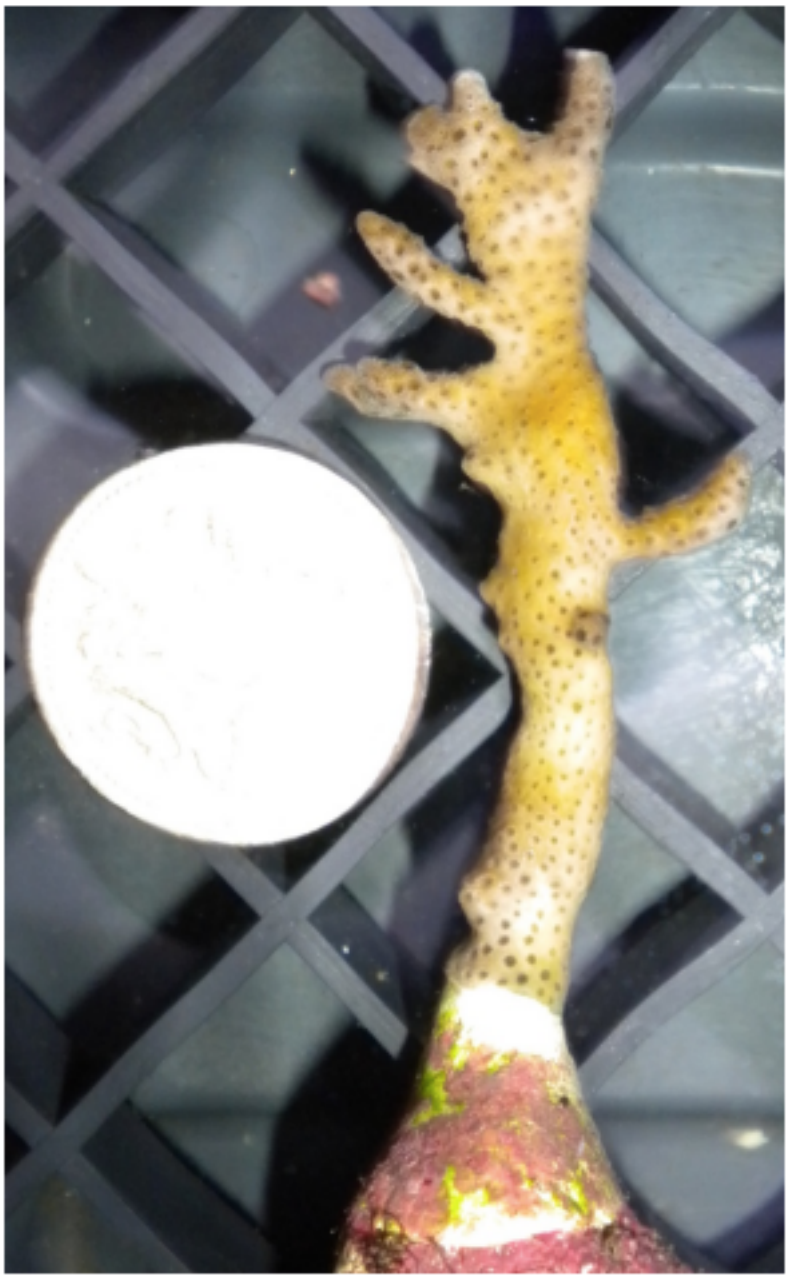


Figure 3.3: Coral *Montipora digitata*

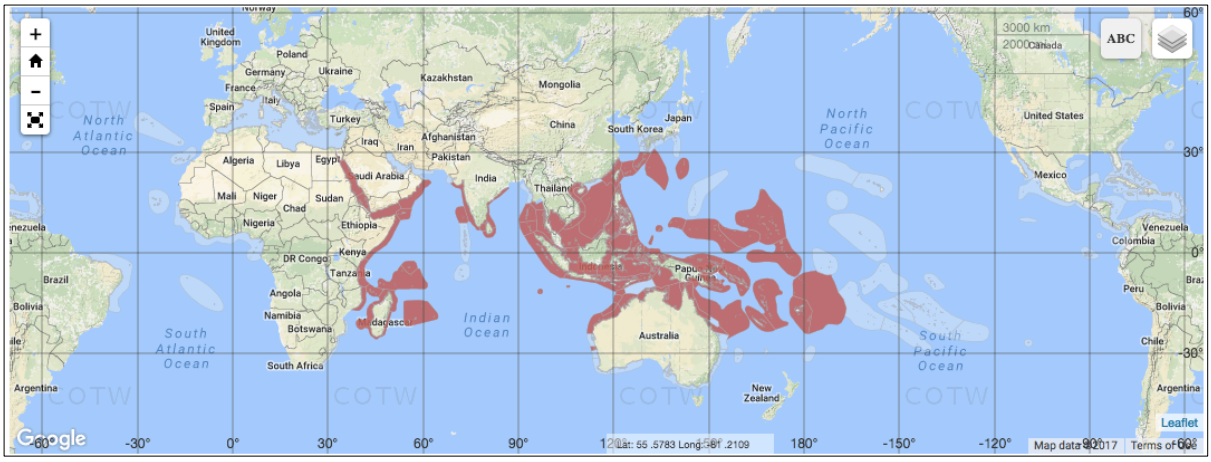


Figure 3.4: Distribution of *Montipora digitata* in the world (www.coralsoftheworld.com)

Montipora digitata (Dana, 1846), belong to the Acroporidae family. Their colonies are digitate or arborescent with anastomosing upright branches (Veron, 2017). The corallites are immersed and small, especially in colonies from typical shallow water. The colours are pale cream or brown, sometimes pink or blue (Veron, 2017) depending on type of irradiance they receive daily (www.marinespecies.com). This species is found in Eastern Africa, Madagascar, Seychelles, Mauritius, Chagos Archipelago, northern Maldives, western India, Sri Lanka, Myanmmar, Thailand, Southeast Asia, Vietnam, southern Japan, Papua New Guinea, southwest to northeast Australia, Solomon Islands, Vanuatu, New Caledonia, Palau, Micronesia, Marshall Islands and Fiji (DeVantier et al., 2008). The map of Fig. 3.4 has shown the world's distribution of this coral species. One of its native countries is Malaysia (DeVantier et al., 2008). Toda et al. (2007) mentioned that *Montipora-Acropora* sp. was the most common coral community on islands along Peninsular Malaysia.

Montipora digitata is a life form colony which is branching and relatively fast-growing coral species, but highly susceptible to environmental influences (Heyward and Collins, 1985; Damayanti et al., 2011). Based on its branching morphology, *M. digitata* was found to be unaffected by thermal and turbidity stresses on Okinawa Island between 1995 until 2009 (Hongo

and Yamano, 2013). Li et al. (2008) found that branching species such as *Montipora* spp. have lower zooxanthellae density which makes them more susceptible to bleaching, than in massive species such as *Porites* spp. The bleaching susceptibility of coral taxa were based on the clade of the *Symbiodinium* spp, referring to LaJeunesse et al. (2003). They found that *M. digitata* which were considered as 'bleaching-resistant' carried *Symbiodinium* spp. of C15, and distinguished it from most montiporid symbioses that are thermal-stress-sensitive (LaJeunesse *et al.* 2003). Stimson et al. (2002) also reported on the bleaching survivor species of *M. digitata* at Sesoko Beach and categorized it as a low mortality species, comparing the aspects of having higher densities of zooxanthellae per square centimeter and a very low rate of release of degraded zooxanthellae. Moreover, on bleaching susceptibility, in Kenya in 1993, the *Montipora* spp. were the only genera that bleached completely (100% of the colony), while none of the *Acropora* colonies were bleached (Souter and Liana, 2005). The study of Ward et al. (2000) in Heron Island found that mass bleaching of 1998 affected the reproduction of corals of all *Montipora* spp., including *M. digitata.* The bleaching had reduced the energy available to the coral because it had lost a great amount of its symbiotic dinoflagellates. This coral species showed no bleaching response and only minor photochemical changes in its symbiont, in a thermal and light stress experiment at 33°C (Krueger *et al*. 2015). This study compared the response of symbiont and host enzymatic antioxidants in this tolerant coral (Krueger et al. 2015). Yakovleva and Hidaka (2004) studied this coral species at 24°C and 31°C and high light (1200 µmol quanta $m^{-2}s^{-1}$) and it showed that the species had significant reduction in photochemical efficiency (F_v/F_m) at both temperatures. Bleaching is most pronounced in shallow waters and particularly affects fast-growing species including *Montipora* spp. (Jonsson, 2003).

3.3 Coral *Seriatopora hystrix*

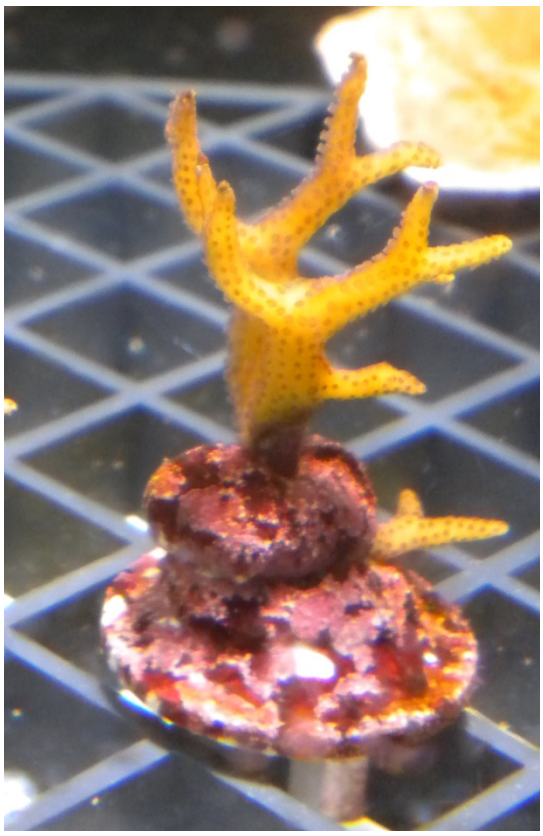


Figure 3.5: Coral *Seriatopora hystrix* for the experiments

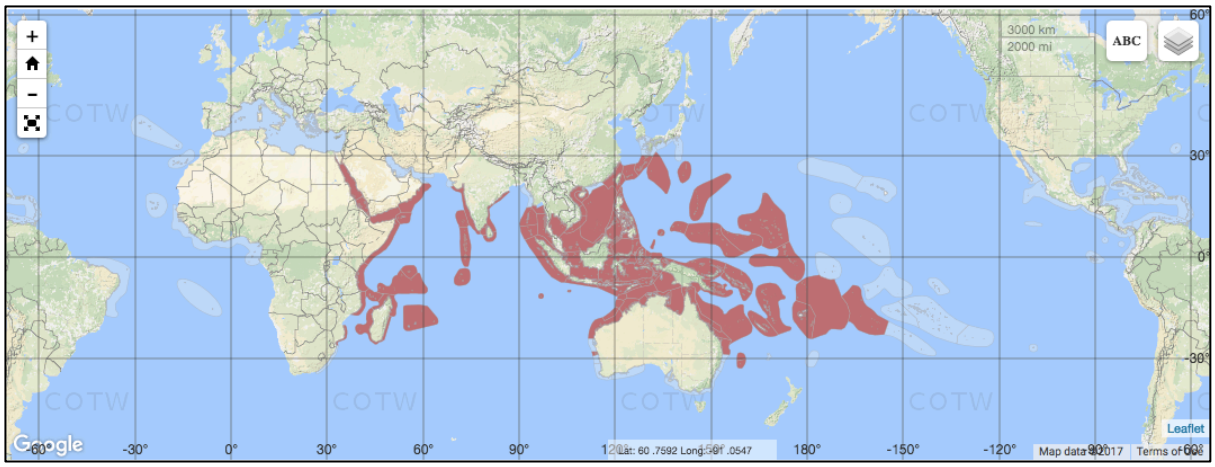


Figure 3.6: Distribution map of *Seriatopora hystrix* in the world (www.coralsoftheworld.com)

Seriatopora hystrix colonies form extensive stands which their branches are thin and tapered to a point; hence they have a needle-like appearance. However, the thickness and length of the branches depend on the wave action in their habitats (Veron, 2017). It can be found in the Indo-West Pacific, Red Sea, Gulf of Aden, the southwest and central Indian Ocean, the central Indo-Pacific, tropical Australia, southern Java and the South China Sea, the oceanic West Pacific and the central Pacific and it is a native species in Malaysia, as shown in Fig. 3.6. (Hoeksema et al., 2014). It is usually found in shallow tropical reef environments, especially intertidal reef flats (Hoeksema et al., 2014). *S. hystrix* is can be found from 3-15 m and deeper from 18-20 m, in the South China Sea (Titlyanov and Titlyanova, 2002) and it can form fields 1m across. (Hoeksema et al., 2014).

Because it is geographically widespread; this coral is a suitable species to examine the processes of ecological diversification and local adaptation of corals, as in the study of Bongaerts *et al.* (2012). *S. hystrix* is known as a thermal-sensitive coral, as in Hoegh-Guldberg and Smith (1989), the corals bleached rapidly when exposed to water temperature above 30°C, which had reduced the expulsion rate of zooxanthellae up to 7-hour exposures. But the corals did not respond toward reduced salinities and sudden increases in solar irradiance (Hoegh-Guldberg and Smith, 1989). When looking at the effects of bleaching at the molecular level, *S. hystrix* may acclimate to fluctuating temperatures by increasing its capacity for photosynthesis (Mayfield *et al.* 2012). The study was focused on the maximum dark-adapted quantum yield of PSII, *Symbiodinium* mRNA and its growth after exposed to stable water temperature of 26°C and 6°C fluctuation over a 12 h period for 7 days. This species had shown no significant differences of photosynthetic quantum yield of shallow 1 m coral and deep 60m coral (Nir et al. 2011). In a study of examining temperature effects on coral's F_v/F_m, it is found that *S. hystrix* corals had a reduction in F_v/F_m during the exposures to temperatures of 22°C, 24°C, 26°C and 28°C (Zartler, 2012).

3.4 Collection and Maintenance of the Corals

In this study, three hard coral species were used for the temperature-light and temperature-nitrate stress experiments. *Stylophora* pistillata, *Montipora digitata* and *Seriatopora hystrix,* have similar morphological structure but different levels of bleaching susceptibility. They were chosen because of their high and moderate susceptibility to bleaching, fast growth, and abundance in Malaysian waters (Mazlan et al., 2005; Loh et al., 2001). The corals nubbins of the species were originally from Sustainable Sources Ltd, a coral supplier situated in York, United Kingdom. The corals were cultured from mother colonies which were in captivity for at least five years. The corals originated from a customs seizure of an illegal shipment from Indonesia, and were maintained in Sustainable Sources culture facility under metal halide lighting (400w lamp, 10 00K) for 8 hours daily, in artificial seawater of salinity between 32-36ppt and temperatures range from 22.5 to 27°C. The corals were not specifically fed in their system (A. Sharp, personal communication, December 1, 2015).

The corals were delivered by courier from York within 12 hours in a polystyrene box, with 3-5 coral nubbins in plastic bags, each filled with warm seawater for a few hours. After being delivered, the corals were acclimatised by placing the plastic bags containing the nubbins into aquaria for 10-15 minutes, to stabilize the water temperatures. Later, corals were put in a large water bucket in their supplied water, to which aquarium tank water was added by dripping it very slowly using a tube (Figure 3.7). The reason for doing this is to let the corals adapt and adjust slowly to the new environment after being shipped. Even a short shipping time in a container can cause the coral to be shocked. From time to time, the water in the bucket was checked for its salinity, temperature and pH to see if the parameters match with the aquarium tank. When done, the corals were transferred into the tank. For the first few weeks, the corals

were placed at mid tank or lower levels, to keep away from direct light. Some corals excreted mucus at the beginning but slowly adapted to the new surroundings. Mucus release is a defence against a multitude of environmental stresses (Brown and Bythell, 2005).

Figure 3.7: Acclimation process for the delivered corals to a new aquarium environment, where corals were put in a large water bucket, filled with the new tank water by dripping it very slowly using a tube.

All of the coral species were maintained in a 100-L aquarium tank with sea water flow-through (3100 L/h), with fresh filtered seawater pumped directly from the Menai Strait. The water temperature was 26-27°C, equivalent to the tropical sea ecosystem. Corals were exposed to light intensity of 200 µmol quanta $m^{-2}s^{-1}$, on a 10h light /14h dark cycle, under a metal halide (HQI-TS 400W; Philips, France). Acrylic covers were used to reduce ultraviolet (UV) radiation, to keep the corals at optimum light, to avoid radiation induced bleaching (Császár et al., 2010). Tanks were cleaned once a week to remove algal growth. Corals were maintained under these conditions prior to the experiments for 1 month for adaptation and stability in a new environment.

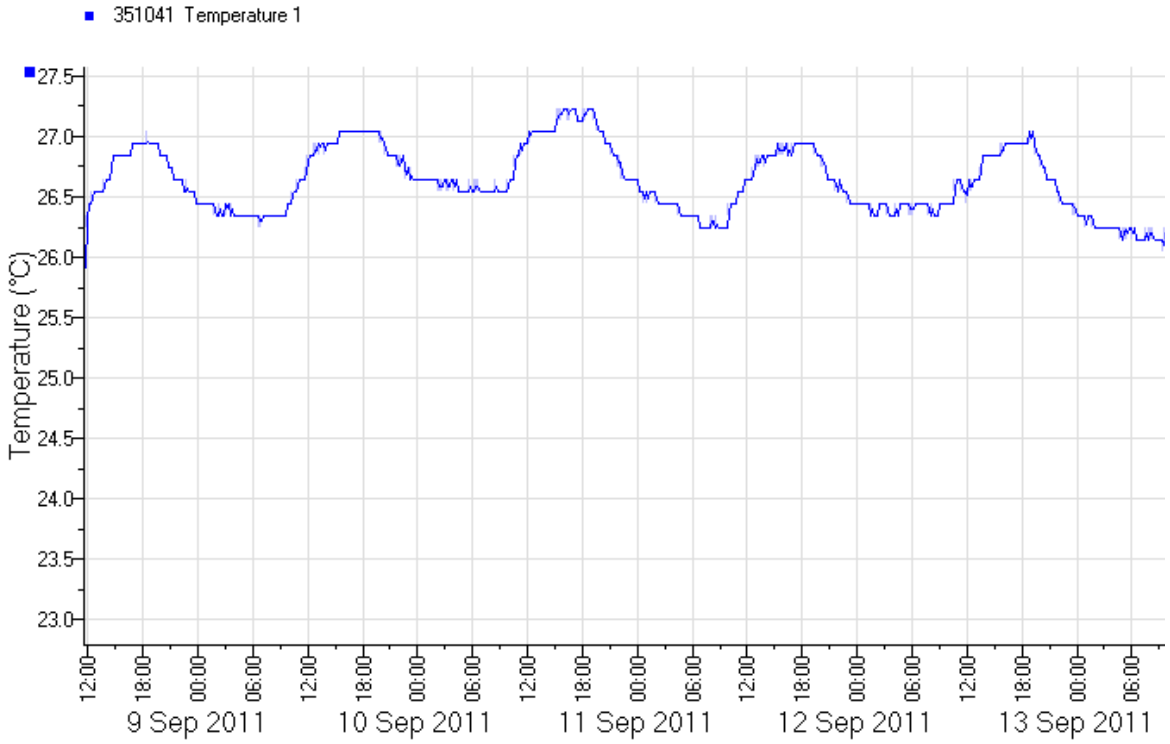


Figure 3.8: Aquarium temperature control during maintenance conditions (day and night) in which temperatures vary by up to 1 degree across a day.

3.5 Experimental tank system

An experimental tank system for this study was designed as in Figure 3.9. Before an experiment started, the heater was set for a specific temperature in the experimental tanks (without putting in the corals yet) and left to stabilise for 3 hours. The temperature in each tank was monitored throughout the experiment to ensure that temperatures remained stable.

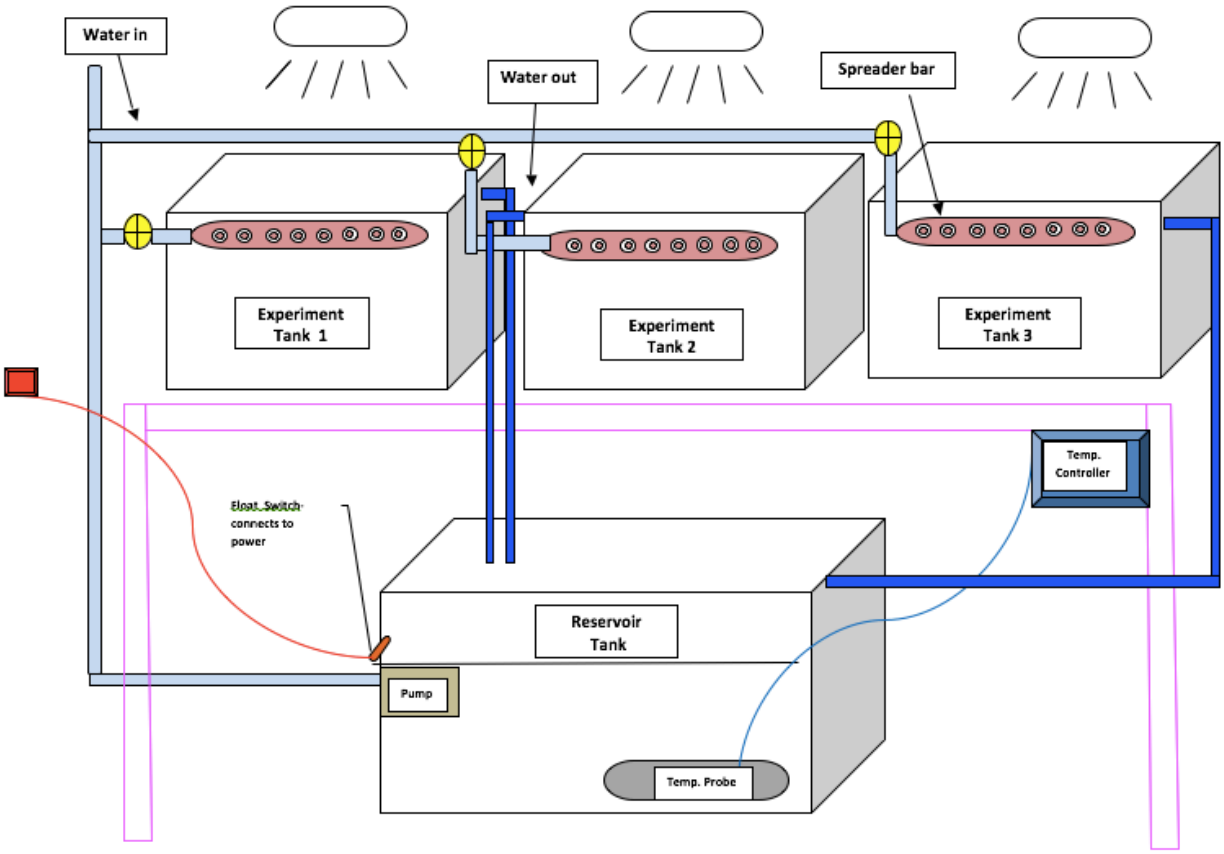


Figure 3.9: Diagram shows the tank system set up for both experiments in this study; Experiment 1: high/ambient temperature and light stress treatments and Experiment 2: high/ambient temperature and nitrate stress treatments for three coral species in a laboratory.

For both experiments, the tank system consisted of 3 aquaria (50 l each) which were used for each treatment as replicates (Figure 3.9). All experimental aquaria were aerated by spreader bars to make sure the water was evenly heated. A strong powerhead pump was used in each aquarium tank to circulate the water. In each aquarium, the coral pieces were placed randomly.

3.6 Coral Husbandary

Aquariums were cleaned by lightly scraping the tank glass every day to get rid of any nuisance algae growth. A few snail *Trochus* sp. were being kept together with the corals to control algae and diatom blooms. Each week, corals were removed from the tanks for growth rate measurement; white tips on the coral nubbins indicated that they are growing. Water quality was checked daily (25-27°C temperature, less than 2ppm nitrate, 0.1 ppm phosphate and 7.7-7.9 pH) and each week a 10 to 20% water partial change was undertaken. During the water changes, corals were not handled to avoid stressing them, but rather the water was siphoned out by using a pipe and the corals kept underneath the water surface throughout the water changes process. Corals were not fed for the husbandry period. The use of water with correct parameters such as salinity, temperature, pH and calcium are the basic concern to maintain healthy corals (Calado et al., 2017). Water flow is an important environmental parameter that can influence the coral quality (Kandorp and Kubler, 2001) and hence a good strong pump (3100 L/h 5W, model NWA3000) was employed.

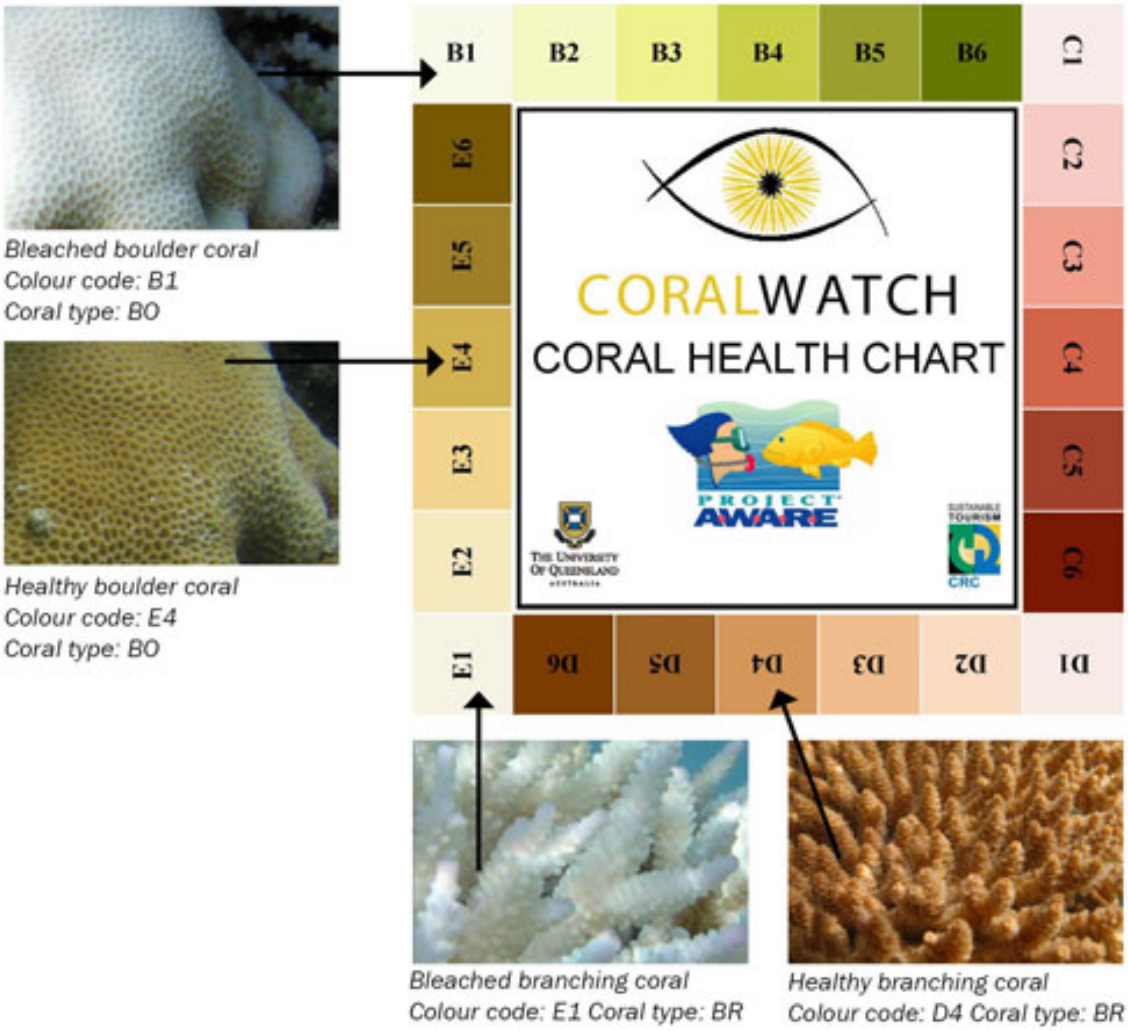


Figure 3.10: CoralWatch Coral Health Chart by CoralWatch.org.

For coral maintenance, the Coral Warch coral health chart was used to identify the healthiness of corals by their colours (Coral Watch, 2008). The colour charts are based on the actual colours of bleached and healthy corals (Coral Watch, 2008). Each of the colour squares on the chart defines the concentration of symbionts contained in the coral tissue, which is linked to the coral's health (Coral Watch, 2008). This standardized colour reference card for corals (Figure 3.10), is an inexpensive, user-friendly technique and can be used for rapid, wide-area assessment of changing coral condition (from normal to bleaching state) without sacrificing the corals (Siebeck et al., 2006); an important consideration here. In this study, the technique of comparing the coral

nubbin colour with the coral health chart is based on Siebeck et al. (2006). For the daily coral's maintenance in this study, the health chart is held near the coral colony and matched with the colour score (1-6). A consistent sampling strategy was used by taking measurements at distance of 3cm from the coral's tip, to avoid colour variations that can be usually found in and around the axial polyp (Siebeck et al., 2006). The CoralWatch Coral Health Chart has a six-point scale which changes in coral colouration (Coral Watch, 2008). A colour score difference larger than 2 score indicates a significant change in symbiont density and chlorophyll *a* content and thus bleaching state (Siebeck et al., 2008). However, during the maintenance and coral colour check, none of the corals showed a difference of 2 scores, which means the corals are maintained in normal and healthy state throughout the maintenance period. In this study, *S. pistillata* exhibits a colour scale of C1 to C6, and the healthy state of this coral species is recorded between score C4-C5. While for *M. digitata* and *S. hystrix*, their colour score is D1 to D6, and their normal healthy colours are scoring as D4, D5 or D6. The average coral colour scale is shown in Figure 3.10, where average colour score for *S. pistillata* is C4, *M. digitata* is C5 and *S. hystrix* is C4.

3.7 Thesis Hypotheses

1. The combined stressors of high-ambient temperature, and light and nitrate levels, may decrease photosynthetic performance of corals measured by the maximum quantum yield of chlorophyll fluorescence (F_v/F_m) for all the coral species of *S. pistillata, M. digitata and S. hystrix*.
2. Changes in photosynthetic capacity of the corals species may be seen in the recorded maximum quantum yield of experimented corals before and after stress. A decrease in photosynthetic capacity can be caused by photoinhibition which interrupts the photosynthetic activities for growth and energy flow.
3. Coral species may be changed in their physical appearance in terms of their pale colouration and mortality level for highly susceptible corals like *M. digitata* and thermal-sensitive corals like *S. hystrix.* The physical appearance changes are related to the corals morphology.

3.8 Thesis Objectives

1. To measure the photosynthetic performance of corals by recording maximum quantum yield of chlorophyll fluorescence (F_v/F_m) in *S. pistillata, M. digitata and S. hystrix* in stress treatments of (a) ambient (27°C, 200 µmol quanta $m^{-2}s^{-1}$), (b) high light (27ºC, 520 µmol quanta $m^{-2}s^{-1}$), (c) high temperature (30°C, 200 µmol quanta $m^{-2}s^{-1}$) and (d) high temperature + high light (30°C, 520 µmol quanta $m^{-2}s^{-1}$).
2. To record the maximum quantum yield of chlorophyll fluorescence (F_v/F_m) of coral species; *S. pistillata, M. digitata and S. hystrix* in stress treatments of (a) ambient (27°C, 200 µmol quanta $m^{-2}s^{-1}$), (b) high light (27ºC, 520 µmol quanta $m^{-2}s^{-1}$), (c) high

temperature (30°C, 200 µmol quanta $m^{-2}s^{-1}$) and (d) high temperature + high light (30°C, 520 µmol quanta $m^{-2}s^{-1}$); to assess the coral's resilience towards the stressors.

3. To differentiate changes in quantum yield fluorescence among the three corals species; *S. pistillata, M. digitata and S. hystrix* before stress, after stress and after 24-h recovery stage.
4. To record the changes in physical appearance (paling and colour) of each coral species before and after the stress treatments; to relate the F_v/F_m with the physical appearance corals.

References

Atkinson M.J., Carlson B. and Crow G.L., 1995. Coral growth in high-nutrient, low-pH seawater: a case study of corals cultured at the Waikiki Aquarium, Honolulu, Hawaii. *Coral Reefs* **14(4):** 215-223.

Baohua Z., Guangce W., Bo H. and Tseng C.K. 2004. Effects of temperature, hypoxia, ammonia and nitrate on the bleaching among three coral species. *Chinese Science Bulletin* **49 (2):** 1923-1928.

Bhagooli R. and Hidaka M. 2003. Comparison of Stress Susceptibility of *in hospite* and Isolated Zooxanthellae among Five Coral Species. 2003. *Journal of Experimental Marine Biology and Ecology* **291:** 181-197.

Bhagooli R. and Hidaka M. 2004. Photoinhibition, bleaching susceptibility and mortality in two scleractinian corals, *Platygyra ryukyuensis* and *Stylophora pistillata*¸ in response to thermal and light stresses. *Comparative Biochemistry and Physiology Part A* **137**: 547-555.

Brown B.E. and Bythell J.C. 2005. Perspective on Mucus Secretion in Reef Corals. *Marine Ecology Progress Series* **296:** 291-309.

Calado R., Olivotto I., Oliver M.P. and Holt G.J. 2017. Marine ornamental species aquaculture. John Wiley & Sons Ltd. United Kingdom.

Csaszar N.B., Ralph P.J., Frankham R., Berkelmans, R. and van Oppen M.J. 2010. Estimating the potential for adaptation of corals to climate warming. *PLoS One* **5 (3):** e9751.

CoralWatch. 2008. Online website and database of bleaching observations http://www.coralwatch.org.

Damayanti L.P.A., Ahyadi H., Candri D.A. and Sabil A. 2011. Growth rate of *Acropora Formosa* and *Montipora digitata* transplanted in Biorock in Gii Trawangan. *Journal of Indonesia Coral Reefs* **1 (2):** 114-119.

DeVantier L.M., De'Ath, G., Turak E., Done T.J. and Fabricius, K.E. 2006. Species richness and community structure of reef-building corals on the nearshore Great Barrier Reef. *Coral reefs* ***25(3):*** 329-340.

Franklin D.J., Hoegh-Guldberg O., Jones R.J. and Berges J.A. 2004. Cell death and degeneration in the symbiotic dinoflagellates of the coral *Stylophora pistillata* during bleaching. *Marine Ecology Progress Series* **272:** 117-130.

Fitt W.K., Gates R.D., Hoegh-Guldberg O., Bythell J.C., Jatkar A., Grottoli A.G., Gomez M., Fisher P., Lajuenesse T.C., Pantos O., Iglesias-Prieto R., Franklin D.J., Rodrigues L.J., Torregiani J.M., van Woesik R. and Lesser M.P. 2009. Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata*, to short-term thermal stress: The host does matter in determining the tolerance of corals to bleaching. *Journal of Experimental Marine Biology and Ecology* **373:** 102-110.

Ferrier-Pagès C., Gattuso J.-P. And Jaubert J. 1999. Effect of small variations in salinity on the rates of photosynthesis and respiration of the zooxanthellae coral *Stylophora pistillata*. *Marine Ecology Progress series* **181:** 309-314.

Franklin D.J., Molina Cedrés C.M. and Hoegh-Guldberg O. 2006. Increased mortality and photoinhibition in the symbiotic dinoflagellates of the Indo-Pacific coral *Stylophora pistillata* (Esper) after summer bleaching. *Marine Biology* **149:** 633-642.

Grover R., Maguer J-F., Allemand D. and Ferrier-Pagès C. 2003. Nitrate uptake in the Scleractinian coral *Stylophora pistillata*. *Limnology and Oceanography* **48** (6): 2266-2274.

Harborne A., Fenner D., Barnes A., Beger M., Harding S. and Roxburgh T. 2000. Status report on the coral reefs of the east coast of Peninsula Malaysia. *Report by Coral Cay Conservation Ltd.*

Heyward A.J. and Collins J.D., 1985. Fragmentation in *Montipora ramosa*: the genet and ramet concept applied to a reef coral. *Coral Reefs* **4 (1):** 35-40.

Hoegh-Guldberg, O. and Smith, G.J., 1989. The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. *Journal of Experimental Marine Biology and Ecology* **129 (3):** 279-303.

Hoeksema B.W., Rogers A. and Quibilan M.C. 2014. *Stylophora pistillata.* The IUCN Red List of Threatened Species 2014. Downloaded on 01 February 2017.

Hongo C. And Yamano H. 2013. Species-specific responses of corals to bleaching events on anthropogenically turbid reefs on Okinawa Island, Japan, over 15-year period (1995-2009). *PLoS ONE* **8 (4):** 360952.

Jonsson D. 2003. An inventory of coral reefs in Langkawi Archipelago, Malaysia – Assessment and impact study of sedimentation. *Undergraduate Thesis University of Sweden.*

Kaandorp. J.A. and Kubler J.E. 2001. *The algorithm beauty of seaweeds, sponges and corals.* Springer Science and Business Media. Berlin Heidelberg, Germany.

Keshavmurthy S., Hsu C.-M., Kuo C.-Y., Meng P.-J., Wang J.-T. And Chen, C. A. 2012. Symbiont communities and host genetic structure of the brain coral *Platygyra verweyi*, at the outlet of a nuclear power plant and adjacent areas. *Molecular Ecology*: 21.

Koren S., Dubinsky Z. and Chomsky O. 2008. Induced bleaching of *Stylophora pistillata* by darkness stress and its subsequent recovery. *Proceedings of the 11th International Coral Reef Symposium, Florida, 7-11 July 2008.*

Krueger T., Hawkins T.D., Becker S., Pontasch S., Dove S., Hoegh-Guldberg O., Leggat W., Fisher P.L. and Davy S.K. 2015. Differential coral bleaching – contrasting the activity and response of enzymatic antioxidants in symbiotic partners under thermal stress. *Comparative Biochemistry and Physiology* **Part A 190:** 15-25.

LaJeunesse T.C., Loh W.K.W., Van Woesik R., Hoegh-Guldberg O, Schmidt G.W. and Fitt W.K. 2003. Low symbiont diversity in Southern Great Barrier Reef Corals, relative to those of the Carribean. *Limnology and Oceanograhy* **48 (5):** 2046-2054.

Li S., Yu K.F, Shi Q., Chen T.R, Zhao M.X. and Zhao J.X. 2008. Interspecies and spatial diversity in the symbiotic zooxanthellae density in corals from northern South China Sea and its relationship to coral reef bleaching. *Chinese Science Bulletin* **53(2):** 295-303.

Loh W.K.W., Loi T., Carter D. and Hoegh-Guldberg O. 2001. Genetic variability of the symbiotic dinoflagellates from the wide-ranging coral species *Seriatopora hystrix* and *Acropora longicyathus* in the Indo-West Pacific. Marine Ecology Progress Series **222:** 97-107.

Mazlan A.G., Zaidi C.C., Wan-Lofti W.M. and Othman B.H.R. 2005. On the current status of coastal marine biodiversity in Malaysia. *Indian Journal of Marine Sciences* **34 (1):** 76-87.

Nir O., Gruber D.F., Einbinder S., Kark S. and Tchernov D. 2011. Changes in scleractinian coral *Seriatopora hystrix* morphology and its endocellular Symbiodinium characteristics along a bathymetric gradient from shallow to mesophotic reef. *Coral Reefs* **30 (4):** 1089.

Putnam H.M., Edmunds P.J. and Fan T.Y. 2008. Effect of temperature on the settlement choice and photophysiology of larvae from the reef coral *Stylophora pistillata*. *Biology Bulletin* **215:** 135-142.

Riegl B. and Velimirov B. 1991. How many damaged corals in Red Sea reef systems? A quantitative survey. *Hydrobiologia* **216/217:** 249-256.

Siebeck, U. E., Marshall, N. J., Klüter, A., & Hoegh-Guldberg, O. 2006. Monitoring coral bleaching using a colour reference card. *Coral Reefs* **25 (3):** 453-460.

Siebeck, U. E., Logan, D., & Marshall, N. J. 2008. CoralWatch: a flexible coral bleaching monitoring tool for you and your group. In *11ICRS: 11th International Coral Reef Symposium 2008* **Vol. 1:** 549-553. National Coral Reef Institute.

Sharp A. 2015. Personal communication. 1 December.

Souter D. and Lindén O. 2005. Coral reef degradation in the Indian Ocean. CORDIO, Kalmar, Sweden.

Stimson J., Sakai K. and Sembali H. 2002. Interspecific comparison of the symbiotic relationship in corals with high and low rates of bleaching-induced mortality. *Coral reefs.* **21:** 409-421.

Sven Beer & Lennart Axelsson (2004) Limitations in the use of PAM fluorometry for measuring photosynthetic rates of macroalgae at high irradiances, *European Journal of Phycology*, 39:1, 1-7.

Toda, T., Okashita, T., Maekawa, T., Alfian, B. A. A. K., Rajuddin, M. K. M., Nakajima, R., and Terazaki, M. 2007. Community structures of coral reefs around Peninsular Malaysia. *Journal of Oceanography* **63 (1):** 113-123.

Titlyanov E., Bil' K., Fomina I., Titlyanova T., Leletkin V., Eden N., Malkin A. and Dubinsky Z. 2000. Effects of dissolved ammonium addition and host feeding with *Artemia salina* on photoacclimation of the hermatypic coral *Stylophora pistillata. Marine Biology* **137:** 463-472.

Titlyanov E.A. and Titlyanova T.V. 2002. Reef-building corals—symbiotic autotrophic organisms: 1. General structure, feeding pattern, and light-dependent distribution in the shelf. *Russian Journal of Marine Biology* **28:** S1-S15.

Veron J.E.N., Stafford-Smith M.G., Turak E. and DeVantier L.M. 2017. *Corals of the world.* Accessed 2 August 2017, version 0.01 (Beta). http://coralsoftheworld.org/v0.01(Beta).

Ward S., Harrison P., and Hoegh-Guldberg O. 2000. Coral bleaching reduces reproduction of scleractinian corals and increases susceptibility to future stress. *Proceedings 9th International Coral Reef Symposium*. Bali Indonesia.

Wiedenmann J., D'Angelo C., Smith E.G., Hunt A.N., Legiret F-E., Postle A.D. and Achterberg E.P. 2012. Nutrient enrichment can increase susceptibility of reef corals to bleaching. *Nature Climate Change.*

Winter G., Loya Y., Röttgers R. and Beer S. 2003. Photoinhibition in shallow-water colonies of the coral *Stylophora pistillata* as measured *in-situ*. *Limnology Oceanography* **48 (4):** 1388-1393.

Yakovleva I. and Hidaka M., 2004. Differential recovery of PSII function and electron transport rate in symbiotic dinoflagellates as a possible determinant of bleaching susceptibility of corals. *Marine Ecology Progress Series* **268:** 43-53.

Yuen Y.S., Nakamura T., Yamazaki S.S. and Yamasaki H. 2008. Long-term effects of inorganic nitrogen enrichment on the reef-building corals *Stylophora pistillata* and *Acropora* spp. *Proceedings of the 11th International Coral Reef Symposium, Florida 7th-11th July 2008.*

Chapter 4

An analysis of survey results of corals from Reef Check Malaysia and NOAA

4.1 Introduction

This chapter investigates the historical pattern of coral bleaching events in 2009 until 2011 using the Reef Check Malaysia database and NOAA Coral Reef Watch's operational twice-weekly global 50-km satellite coral bleaching monitoring products. This approach should reveal the pattern of bleaching episodes of corals against the bleaching predictions and reports from remote sensing data of NOAA.

Since 1990s, mass coral bleaching events has been reported. These events occur due to anomalously warm ocean water and significantly contribute to degradation of coral reefs ecology (Liu et al., 2014). Every ocean varies in their peak time of bleaching, for instance July until September for northern Atlantic and Pacific Oceans, January until March for the southern Atlantic and Pacific Oceans, April until June for the northern Indian Ocean and January until April for the southern Indian Ocean (Liu et al., 2014).

The Reef Check data and relates it with 50-km monitoring product of Degree Heating Week Coral Reef Watch for 2009 until 2011 in Peninsular Malaysia and East Malaysia. The hypothesis of this work is that satellite data and *in situ* coral reefs substrate survey can be useful predicting bleaching for Malaysian coral reefs.

4.1.1 Reef Check Malaysia

Since 2001, Reef Check Malaysia has been monitoring coral reefs to assess their health in selected islands all over Malaysia (ReefCheck, 2009). Involving volunteer recreational divers and marine scientists, Reef Check undertook 50 surveys in Peninsular Malaysia and 65 surveys in various parts of Sabah and Sarawak, East Malaysia. By using the concept of 'indicator species', data from the monitoring methodology helped scientists and managers to keep track of the changes of reef health every year (ReefCheck, 2009). The "Indicator species" were the marine organisms on the reef living amongst corals and were identified to provide information on the coral reef's health (Chelliah et al., 2014). The Reef Check surveys are conducted along two depths of 3m to 6m and 6m to 12m. They set up a 100 m transect line, and four 20 m transects were surveyed. There are four replicates per transect (8 per complete survey) for statistical data analysis (ReefCheck, 2015).

The regular basic monitoring methodology can give early warning for any deleterious reef changes (mostly by human impacts), and give opportunities to managers to carry out any management actions to save the corals (Chelliah et al., 2014). Reef Check monitoring methodology surveys can infer that the corals' degradation was caused by varying levels of local threats (such as tourism and destructive fishing methods).

Table 4.1: The threats to corals and coral reefs in Malaysia in 2010

Threat	Western Coast of Peninsular Malaysia	Eastern Coast of Peninsular Malaysia	Sabah and Sarawak
Fishing intensity	4	3	5
Fishing damage	3	3	5
Fish blasting	2	2	4
Gleaning	2	1	3
Boat scouring	2	3	4
Population pressure	4	3	4
Sedimentation	5	3	3
Domestic and agriculture pollution	3	2	4
Industrial pollution	3	1	1
Oil spill	2	1	2
Disease and predation	2	4	3
Dredging	2	1	2
Coral mining	1	1	3
Tourist activities	1	2	2
Coral bleaching	1	1	1

Scale:

1 = none to rare; 2 = very low concentration; 3 = some damage; 4 = medium to high damage; 5 = very high, high stress, very damaging.

Source: Asian Development Bank, 2016.

4.1.2 NOAA Coral Reef Watch (NOAA CRW)

The NOAA Coral Reef Watch (CRW) program's satellite data give the current reef environmental conditions to identify areas at risk of coral bleaching (http://coralreefwatch.noaa.gov). CRW is a product of the U.S. National Oceanic and Atmospheric Administration (NOAA) which uses satellites to record twice weekly 50-km Bleaching Alert Area (https://coralreefwatch.noaa.gov/satellite/baa.php) based on sea surface temperature (SST) monitoring. CRW is a long-term monitoring of physical environmental conditions of coral reef ecosystems worldwide (Liu et al., 2013). These satellite thermal stress monitoring products are a suite for predicting coral bleaching, including: SST, SST Anomaly, Coral Bleaching HotSpots, coral bleaching DHW, Bleaching Alert Area, Virtual Stations and a Satellite Bleaching Alert (SBA) email system (Liu et al., 2013). The data and satellite images can be freely accessed online at: http://coralreefwatch.noaa.gov.

Satellite remote sensing gives synoptic views of worldwide oceans in near-real-time and can monitor remote reef areas (NOAA, 2011). In 1977, NOAA had produced web-acessible, satellite derived, global near-real-time nighttime sea surface temperature (SST) to alert on coral bleaching condition and can also assess the bleaching intensity (NOAA, 2011). The SST product is a twice-weekly night-time sea surface temperature field based on 50km resolution measurements from the Advanced Very High-Resolution Radiometer (AVHRR) on a NOAA polar orbiting satellite (NOAA, 2011). The 50-km resolution data are the average of multiple temperature observations, weighted by distance from pixel center, out to a maximum of 150km (NOAA, 2011).

Degree Heating Weeks (DHW) indicate the accumulation of thermal stress that coral reefs experienced over the past 12 weeks (NOAA, 2013). DHW generated satellite bleaching warnings and alerts are a 12-week accumulated HotSpot which are greater than 1°C. It is accumulated data for up to 3 months and the most current product update. Developed in 2000, it shows that more than 1°C of seawater temperature, above the mean summertime maximum, can cause thermal stress to corals, which possibly can lead to bleaching or mortality.

This chapter is to study the Reef Check data and relates it with 50-km monitoring product of Degree Heating Week Coral Reef Watch for 2009 until 2011 in Peninsular Malaysia and East Malaysia. The hypothesis of this work is that consist use of satellite data and *in situ* coral reefs substrate survey can be useful predicting bleaching for Malaysian coral reefs.

4.2 Methodology

Reef Checks surveys involved 50 sites in Peninsular Malaysia and 47 in East Malaysia, which involves main islands in Peninsular and four main islands in East Malaysia. The monitoring surveys were conducted along two depth contours (3m to 6m and 6m to 12m depth). Transect line of 100m is used where 20m transects are surveyed and separated by 5m. Four replicates per transects are done and there are 8 per complete survey. List of surveys sites is listed in Appendix.

Two environmental variables to detect coral bleaching were examined for the islands around Peninsular Malaysia and East Malaysia. (i) Sea Surface Temperature (SST) (ii) Degree Heating Week Coral Reef Watch (DHW CRW). The Sea Surface Temperature (SST) database was acquired from the Coral Reef Temperature Anomaly Database (CoRTAD) Version 5. CoRTAD Version 5 is approximately 4km resolution SST data on a weekly time scale from 1982 until 2012, from Pathfinder Version 5.2 (NODE, 2017). In this study, only 2010-2011 data were

downloaded from CoRTAD, using Panoply and CoastWatch software. Panoply software plots geo-referenced data compatible with NOAA CoRTAD datasets in format of netCDF, THREDDS and HDF. CoastWatch software is a free software package for reading the satellite data in HDF format as well. CRW's SST anomaly is produced by subtracting the long-term mean SST from the current value (NOAA, 2017). 2010-2011 data used a spatial resolution of 0.5 degree (50km), twice-weekly updated. The colour range of temperature anomalies displayed on the SST anomaly charts is -5.0 to +5.0 °C. and the images of archived SST anomalies for 2010 and 2011 are available from the CRW website, along with the operational 0.5-degree monthly mean SST climatologies (NOAA, 2017).

Coral bleaching Degree Heating Weeks (DHW) by NOAA Coral Reef Watch (CRW) are accumlulated heat stress indicators of coral bleaching and death. Measured by Coral Bleaching Hotspots greater than 1°C in 12-week period, it is a direct relation of the timing and intensity of coral bleaching. It is a cumulative measurement of the intensity and duration of thermal stress and the unit is °C per week. The scale is from 0 to 16°C –weeks (NOAA, 2017). DHW over 4°C-weeks can cause significant coral bleaching, over 8°C-weeks have been shown to cause widespread bleaching and mortality (NOAA, 2017). Bleaching data between 2010 and 2011 were collated to evaluate the bleaching trend in Peninsular Malaysia and East Malaysia using Annual Reef Check survey and SST-DHW from CoRTAD Version 5.

Table 4.2: Coral bleaching thermal stress levels based on the current values of the NOAA CRW operational 50-km Coral Bleaching HotSpot and Degree Heating Week (DHW) products (www.noaa.gov)

Stress Level	Definition	Potential Bleaching Intesity
No Stress	HotSpot ≤ 0	No Bleaching
Bleaching Watch	$0 <$ HotSpot < 1	
Bleaching Warning	$1 \leq$ HotSpot and $0 <$ DHW < 4	Possible Bleaching
Bleaching Alert Level 1	$1 \leq$ HotSpot and $4 \leq$ DHW < 8	Bleaching Likely
Bleaching Alert Level 2	$1 \leq$ HotSpot and $8 \leq$ DHW	Mortality Likely

Data analysis

Reef check raw data were calculated the mean, standard deviation, standard error and were analysed using One-way ANOVA for its statistical analysis. All data were checked for assumptions of normality and homogeneity of variances.

4.3 Results

4.3.1 Survey analysis from Reef Check monitoring surveys in 2009 and 2010

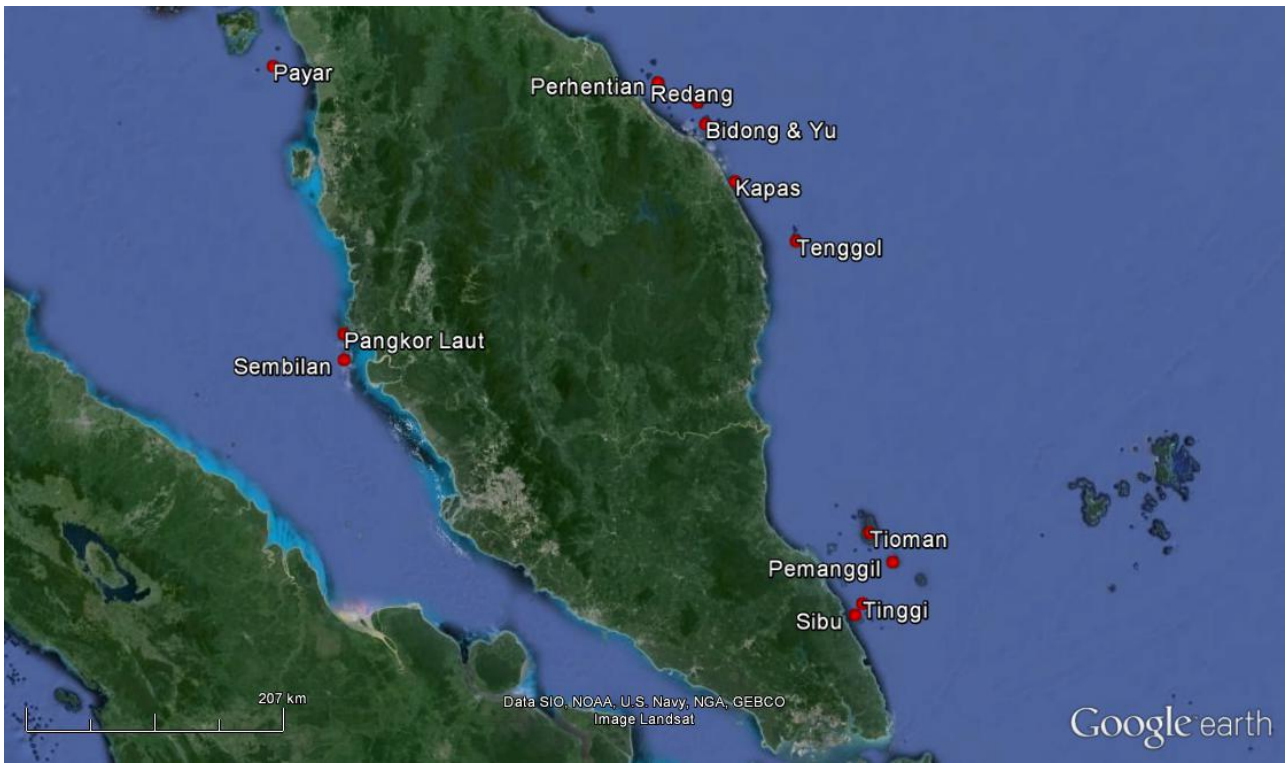


Figure 4.1: Location of surveyed islands in Peninsular Malaysia, by Reef Check Malaysia.

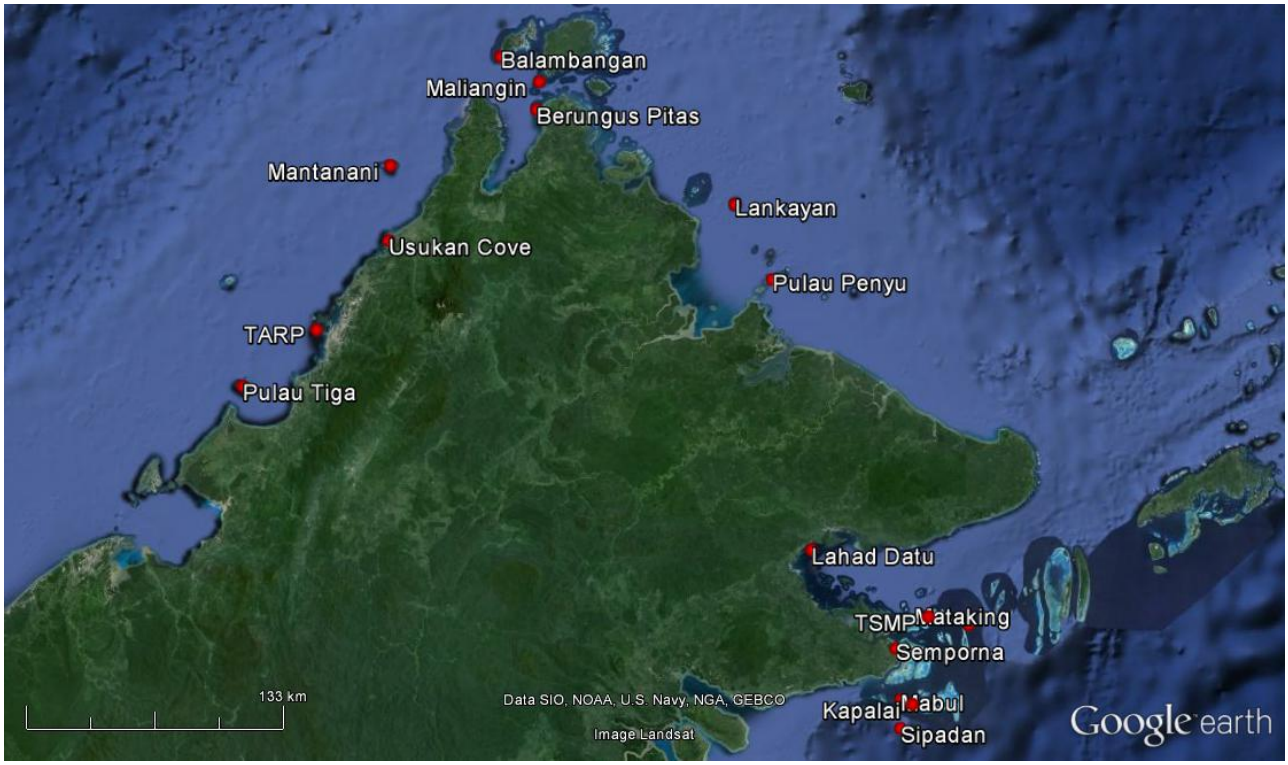


Figure 4.2: Location of surveyed islands in Sabah, East Malaysia, by Reef Check Malaysia.

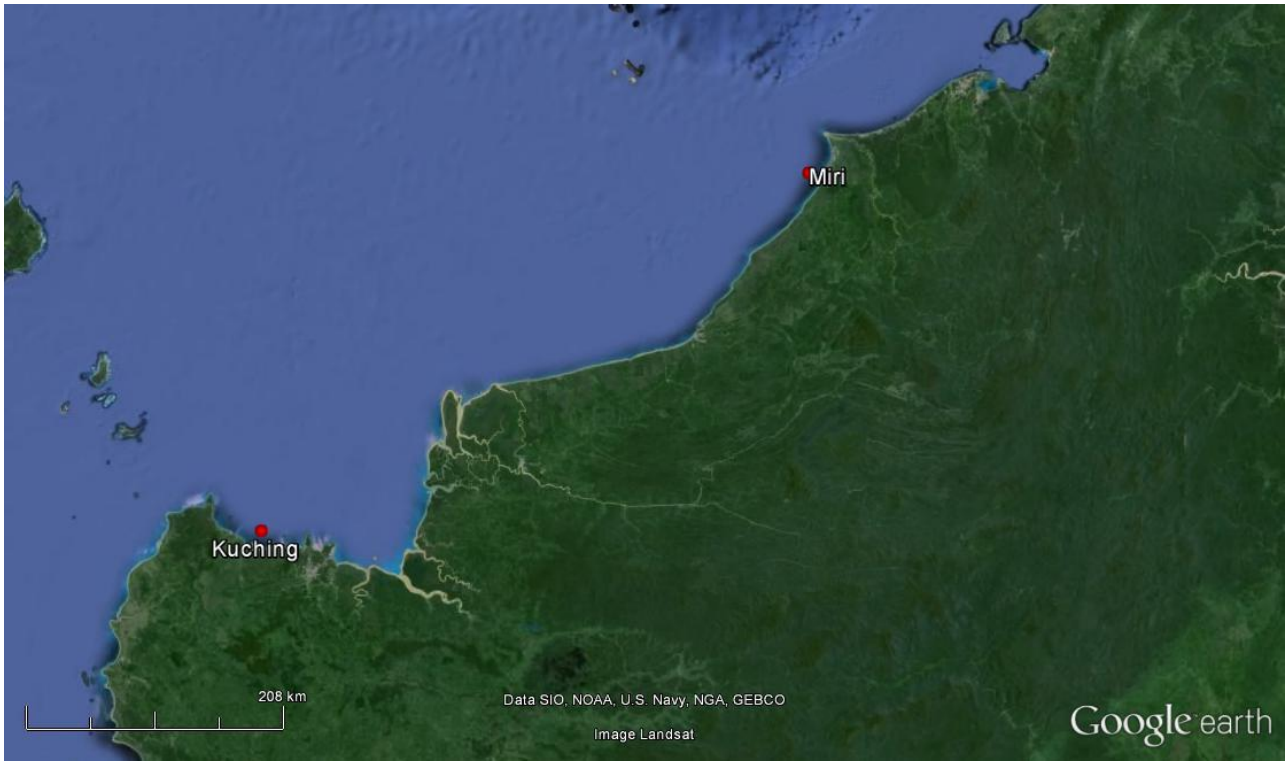


Figure 4.3: Location of surveyed islands in Sarawak, East Malaysia, by Reef Check Malaysia.

In 2009 and 2010, Reef Check had collected data from 50 survey sites in Peninsular Malaysia (Figure 4.2) and 65 in East Malaysia (Figure 4.3 and Figure 4.4). This includes five main islands off Peninsular Malaysia's East Coast inside Marine Protected Areas, which are; Aur, Perhentian, Redang, Tenggol and Tioman islands.

2009 surveys reported that at Perhentian Island, live coral cover (LCC) was found to be slightly reduced by 1% from 2008 to 2009 records and ReefCheck (2009) reported that the reefs were not under serious threat. New hard coral growth was observed at one site of Perhentian, Tanjung Besi, (ReefCheck, 2009). For 2010, Lighthouse, Perhentian Kecil Island shows the highest Reef Killed Coral (RKC) at 22.5%.

In 2009, Redang Island, at the site of Paku Besar Island (off Long Beach), recorded 23% hard coral lost and an increasing Nutrient Indicator Algae (NIA) level from 6.3% to 26.9% (from the year 2006) (ReefCheck, 2009). In Tenggol island, hard cover increased from 34.4 % to 41.4% and NIA declined from 2007 until 2009. In Tioman island, the reef condition improved on Pirate Reef only – elsewhere the hard coral cover decreased by 15%.

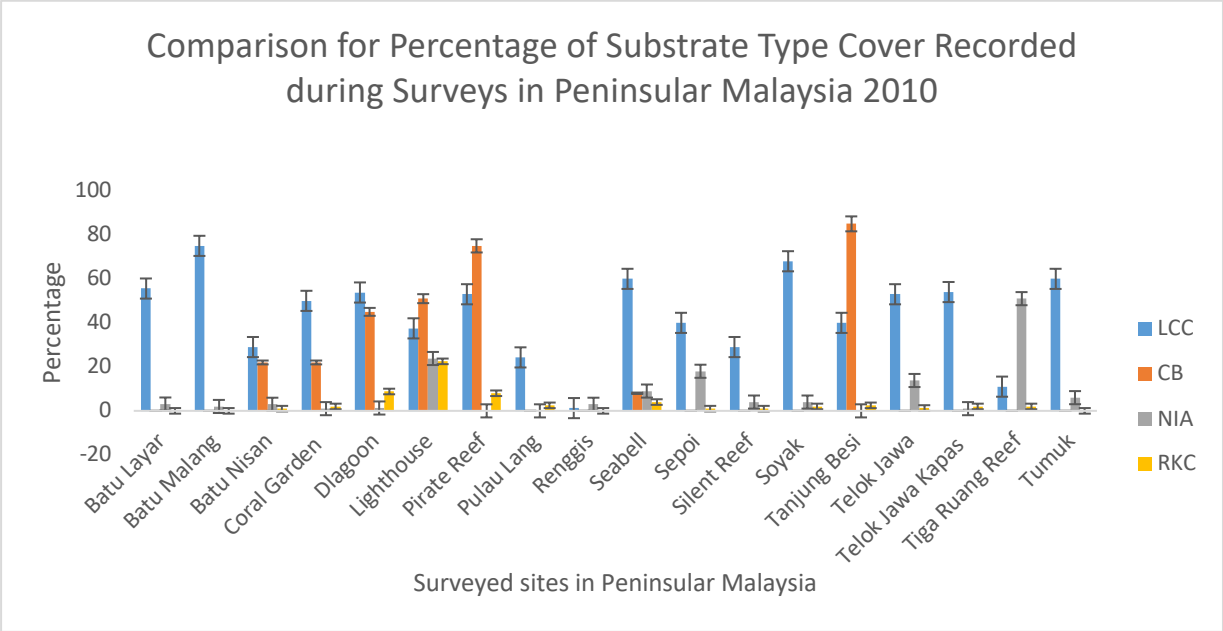


Figure 4.4: Percentage of substrate type (LCC = Live Coral Cover, CB = Coral Bleaching, NIA = Nutrient Indicator Algae, RKC = Recently Killed Coral). The data were recorded during surveys in islands of Peninsular Malaysia in 2010 (between May and July), by Reef Check Malaysia.

Figure 4.4 shows the percentages of Live Coral Cover (LCC), Coral Bleaching (CB), Nutrient Indicator Algae (NIA) and Recently Killed Coral (RKC) of surveyed islands in Peninsular Malaysia in 2010. The list of the site locations is listed in Appendix Table C.1 and C.2. For Peninsular Malaysia in 2010 there are recorded data on coral bleaching compared to 2009 surveys. There were 8 out of 23 site locations with recorded coral bleaching. Tanjung Besi of

Perhentian Island had 85% of bleached corals during the mid June 2010 survey. They did not state the cause of the bleaching in the data, but the 2010 massive coral bleaching event in Malaysia started in mid-April to June (Reef Check, 2012). 75% of coral bleaching was recorded in Pirate Reef, Tioman Island, one of the islands that had the worst bleaching event in June until August 2010 (Reef Resilience, 2014).

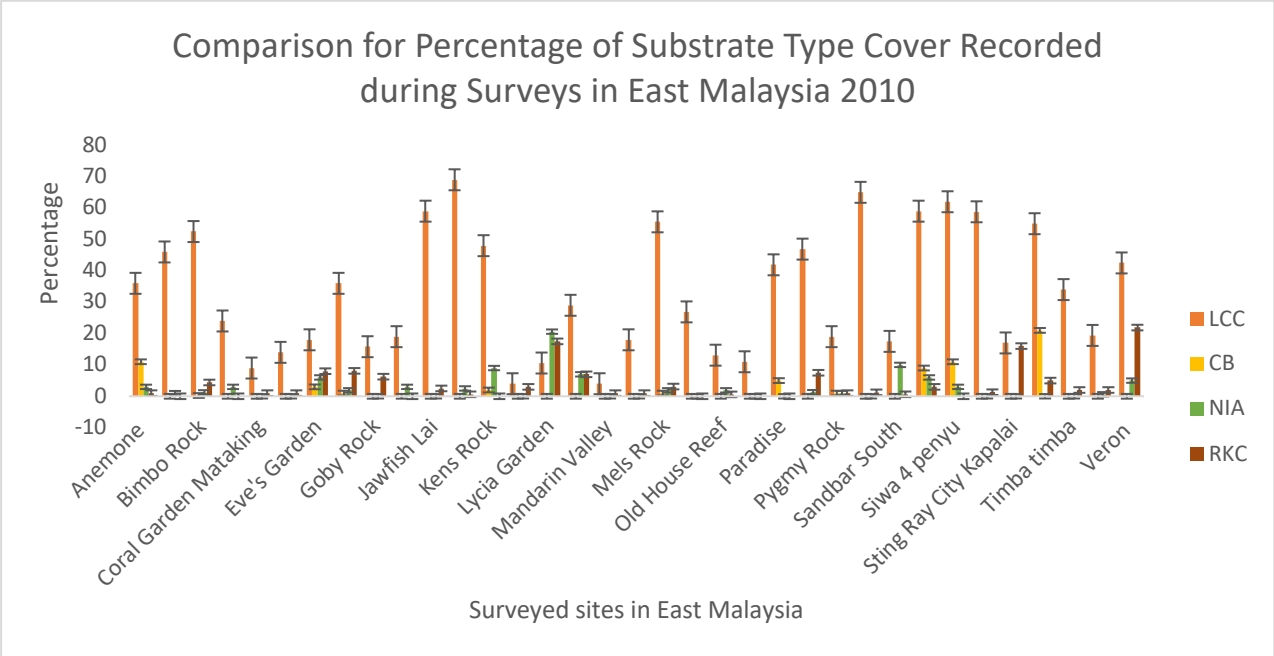


Figure 4.5: Percentage of substrate type (LCC = Live Coral Cover, CB = Coral Bleaching, NIA = Nutrient Indicator Algae, RKC = Recently Killed Coral). The data were recorded during live surveys in islands of East Malaysia in 2010 (between May and July), by Reef Check Malaysia.

In East Malaysia, for 2009, 65 surveys were conducted in Miri, Pulau Gaya, Kudat, Lankayan and Semporna. Only in Miri, the corals were categorized as 'good' (60.5% live coral cover) but in other places is 'fair' condition, based on the Coral Reef Health Criteria. High percentages of Recently Killed Coral (RKC) (average 9%) was the main concern in Lankayan reefs, which reported that Crown of Thorns predations is the cause for the RKC (Reefcheck, 2009).

In 2010, there were records of coral damage by old dynamite fishing and heavy storm damage in a few site locations (Cahaya Way, Coral Garden Mataking, Malohon, Old House Reef). The highest NIA (20.6%) and RKC (7.5%) in 2010 survey is Lycia Garden, situated in Lankayan, Sabah.

4.3.2 Coral bleaching and Degree Heating Weeks in Malaysia for 2009 and 2010

Raw data obtained from Reef Check Malaysia recorded the percentage of coral bleaching, and this was examined along with the coral bleaching alert and degree heating weeks (DHW) products of NOAA to place field observations in context provided by remotely sensed products. 2009 surveys reported low to high bleaching severity from June to September in the whole of Malaysia. The massive coral bleaching event happened between May and July 2010 and bleaching was recorded for Perhentian and Tioman islands in June 2010, by ReefCheck Malaysia (Table 4.3). In Tioman Island, bleaching was 85%, live coral cover was 40%, no NIA was detected on the substrate and 2.5% of recently killed coral was seen. For Tioman island bleaching event, coral mortality was documented (Table 4.4).

For this study, Reef check survey data was correlated with NOAA CRW daily 50-km Sea Surface Temperature (SST) product to identify the highest temperature in Perhentian and Tioman island in June 2010 (Figure 4.6). The temperature was shown to cause bleaching in both islands during the time. Even though the colour pixels were uniform all along the East Coast and West Coast of Peninsular Malaysia; only two islands had coral bleaching during Reef Check Survey data for 2010. Other islands such as Redang, Pangkor and others on the list of site locations of this survey, were believed to have no bleaching event. However, Tan and Heron (2011) reported that there were 54 surveys in Redang island (East Coast of Peninsular Malaysia)

where bleaching was found as deep as 25 meters but none was recorded elsewhere. 75-90% bleaching was reported in Tioman together with Tinggi and Sibu island, East Coast of Peninsular Malaysia (Tun et al., 2010) which Tinggi and Sibu island were not listed as site location for this survey.

CRW Degree Heating Week was extracted from CoRTAD datasets, for Perhentian and Tioman islands during the June 2010 massive bleaching event (Figure 4.7). Perhentian island showed 10-12°C of DHW values and Tioman showed 5-9°C of DHW values. Both locations show DHW values of more than 8°C-weeks, which indicated widespread bleaching and significant mortality (NOAA, 2017).

Table 4.3: Reef Check Malaysia surveys in June 2010 had recorded bleaching corals in four locations of Perhentian and Tioman islands.

Island	**Site location**	**Coral Bleaching (%)**
Perhentian	D'lagoon	45
	Lighthouse	5
	Tanjung Besi	75
Tioman	Pirate Reef	85

Table 4.4: Recorded of mortality corals by species during 2010 mass bleaching event in Tioman island.

Location and year	Coral species	Percentage of coral mortality
Tioman island 2010	*Porites*	46%
	Pocillopora	39%
	Acropora	26%
	Montipora	17%
	Pavona	11%
	Fungia	4%
	Goniastrea	4%
	Platygyra	2%
Perhentian island 2010	No record	

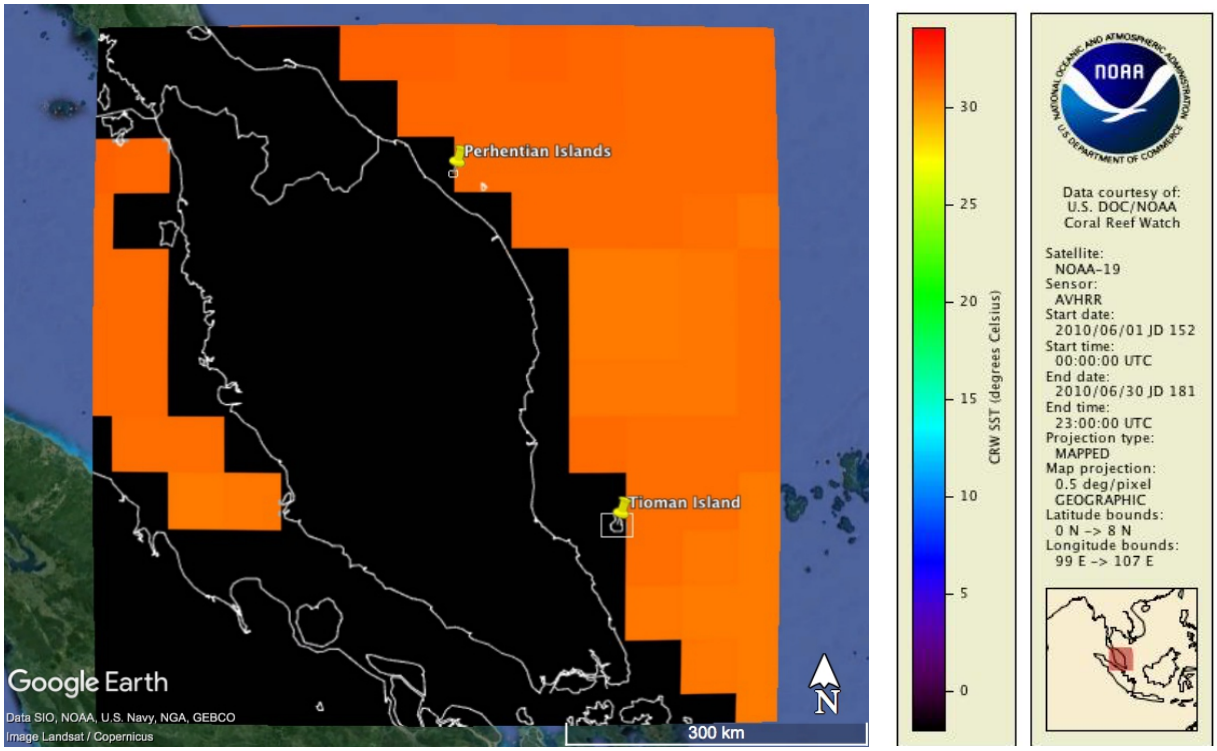


Figure 4.6: Sea surface temperature (SST) for 50km target identifying the temperature in Perhentian and Tioman island in June 2010 was 31°C, by the colour pixel on the image. This image is from NOAA coral reef watch satellite sea surface temperature for 2010 annual maximum composite, extracted from www.noaa.gov and Google Earth.

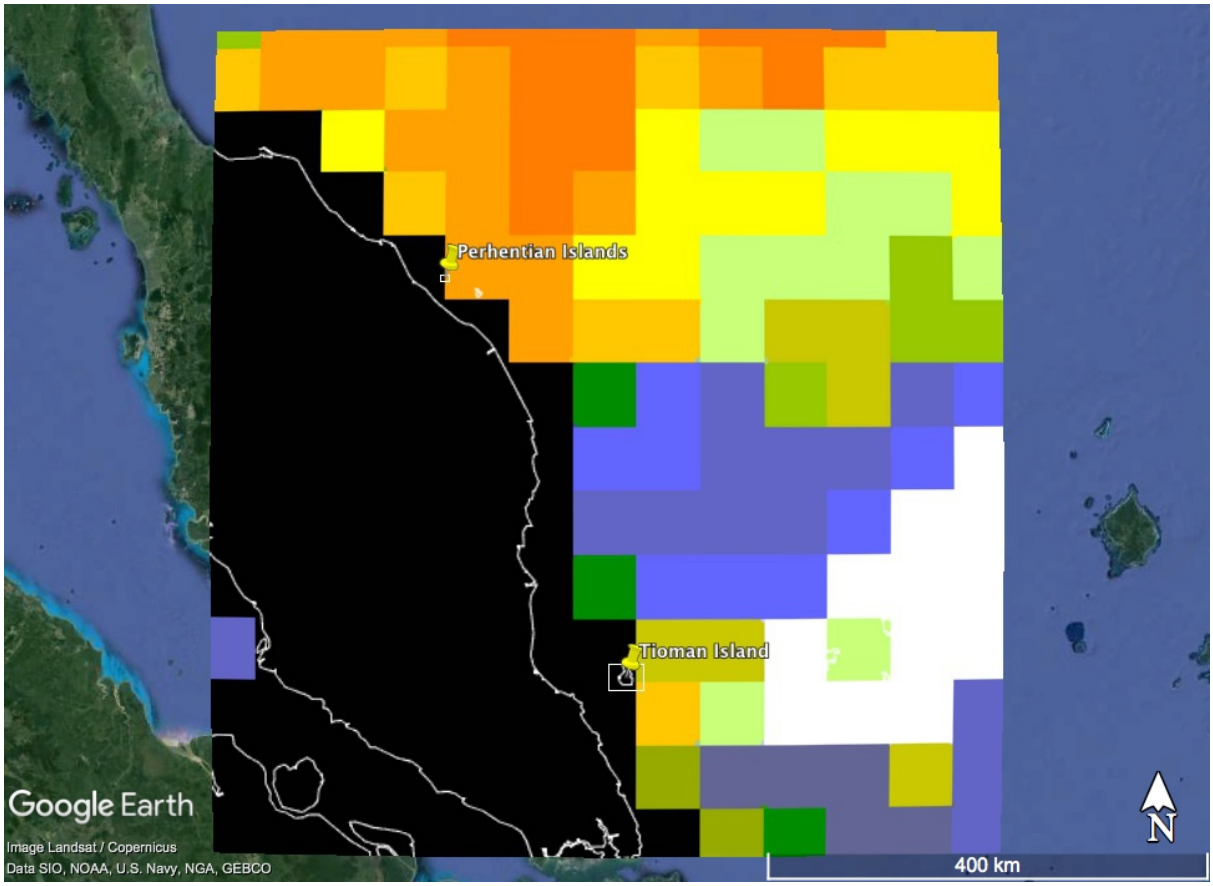


Figure 4.7: CRW Degree Heating Week for Perhentian and Tioman islands during June 2010 massive bleaching event. Perhentian showed 10-12°C of DHW and Tioman showed 5-9°C of DHW. This DHW values equivalent to the reported bleaching at these two locations.

Table 4.5: Coral bleaching survey data in site locations in East Malaysia in 2009

Year	Site location	Coral Bleaching Severity	Coral type	Seawater temperature
2009	Anemone, Miri	Low and medium	Encrusting and plate corals	30°C
	Eve's Garden, Miri	Low	Not mention	30°C
	Unknown location, Miri	High	Encrusting and plate corals	30°C
	Siwa Reef, Miri	Medium	Encrusting and plate corals	29°C
	Sunday Reef, Miri	Medium	Plate corals	30°C
	Santak Reef, Miri	High	Encrusting and plate corals	29°C
	Tukau Reef, Miri	High	Encrusting and plate corals	29°C

For East Malaysia (Table 4.5), 2009 surveys reported low to high bleaching severity in site locations in Miri Sarawak, which mostly affected encrusting and plate corals in 29-30°C water temperature. It occurred during June until September 2009.

This bleaching data in Sarawak was consistent with the NOAA Coral Reef Watch (CRW) twice-weekly 50-km satellite coral bleaching Degree Heating Week (DHW) product. For Sabah state of East Malaysia, Reef Check Malaysia did not have any bleaching corals data for 2009. However, from DHW NOAA, there is a purple colour dot in the satellite image, indicating DHW of 14-16°C-weeks. Reef Check Malaysia may not have been completed for all locations in Sabah.

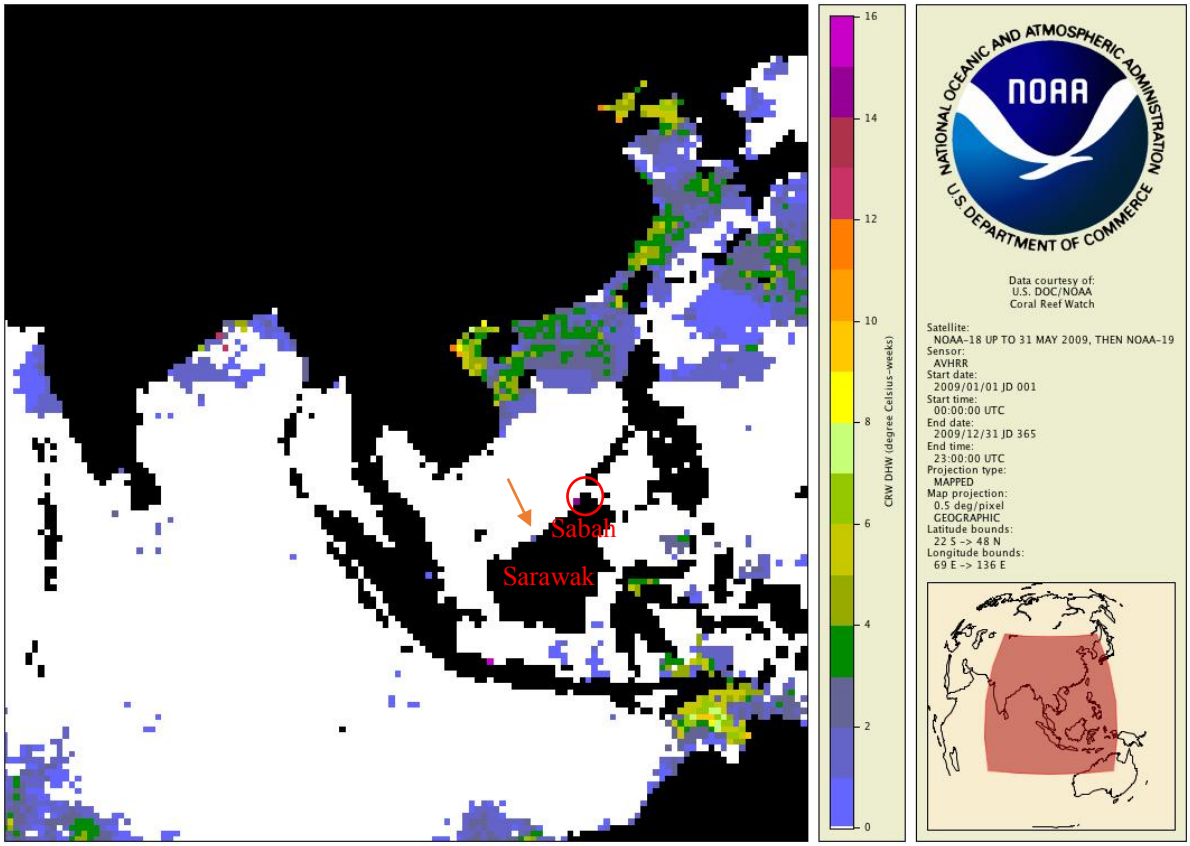


Figure 4.8: Satellite coral bleaching monitoring by Degree Heating Week metric by NOAA Coral Reef Watch (http://coralreefwatch.noaa.gov/satellite) showing the arrow to blue dot in Sarawak and circled purple dot in Sabah, East Malaysia. This image was consistent with the Reef Check Malaysia coral bleaching 2009 survey data for Sarawak, but there was no bleaching data recorded for Sabah.

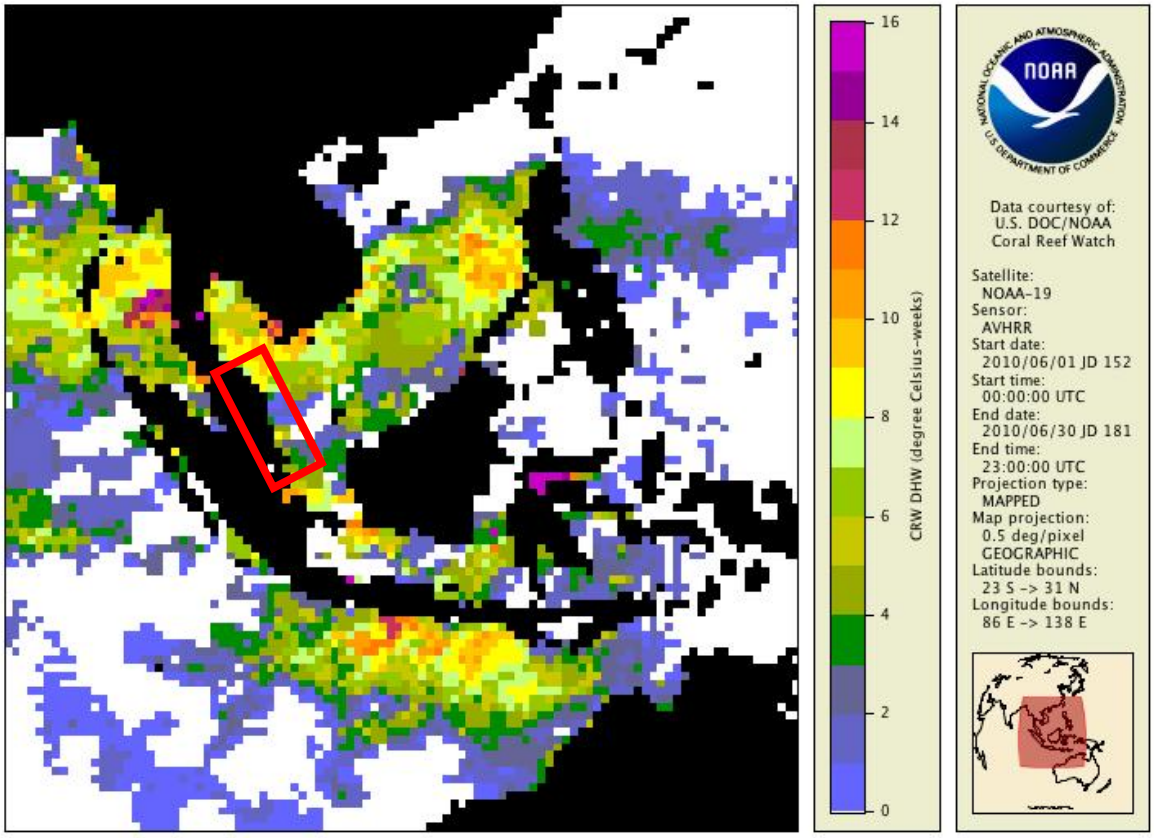


Figure 4.9: NOAA Coral Reef Watch annual maximum satellite coral bleaching Degree Heating Weeks for June 2010. This map is zoomed onto the Malaysian map to show the maximum Degree Heating Week during the 2010 mass bleaching event in Malaysia. Along the East Coast of Peninsular Malaysia, the degree celcius colour pixel was blue to yellow (red box) (2-8°C) and for East Malaysia, the degree celcius colour pixel was blue to dark green (2-4°C).

For East Malaysia, during the 2010 mass bleaching event, coral bleaching was recorded in Miri, Lankayan and Mabul Island, where site location Sunday Reef (in Miri) recorded the highest percentage of bleaching during May-July 2010 (Table 4.6). Figure 4.10 the trend between coral bleaching at the surveyed sites with the data of nutrient indicator algae. For site location Siwa 4, Sunday Reef, Kens Rock and Paradise, there is no recorded NIA but there is still coral bleaching data. It means that the coral bleaching in the area did not affected by the nitrification.

Table 4.6: Reef Check Malaysia surveys in May to July 2010 had recorded bleaching corals in Miri and Lankayan (Sarawak) and Mabul in Sabah. Also recorded are the Live Coral Cover (LCC), Nutrient indicator algae (NIA) and Recently killed coral (RKC) in surveyed sites.

Site location	Coral Bleaching (%)	Live coral cover (%)	Nutrient indicator algae (%)	Recently killed coral (%)
Anemone, Miri	11	36	3	1
Eve's Garden, Miri	3	18	6	8
Siwa 4, Miri	9	59	0	3
Siwa Penyu, Miri	11	62	3	0
Sunday Reef, Miri	21	55	0	5
Bimbo Rock, Lankayan	0.25	52.5	1.25	4.4
Froggie Fort, Lankayan	1	36	1.80	8.1
Kens Rock, Lankayan	1	48.1	0	17.5
Mels Rock, Lankayan	1	55.6	1.9	3.1
Paradise, Mabul	5	41.9	0	0

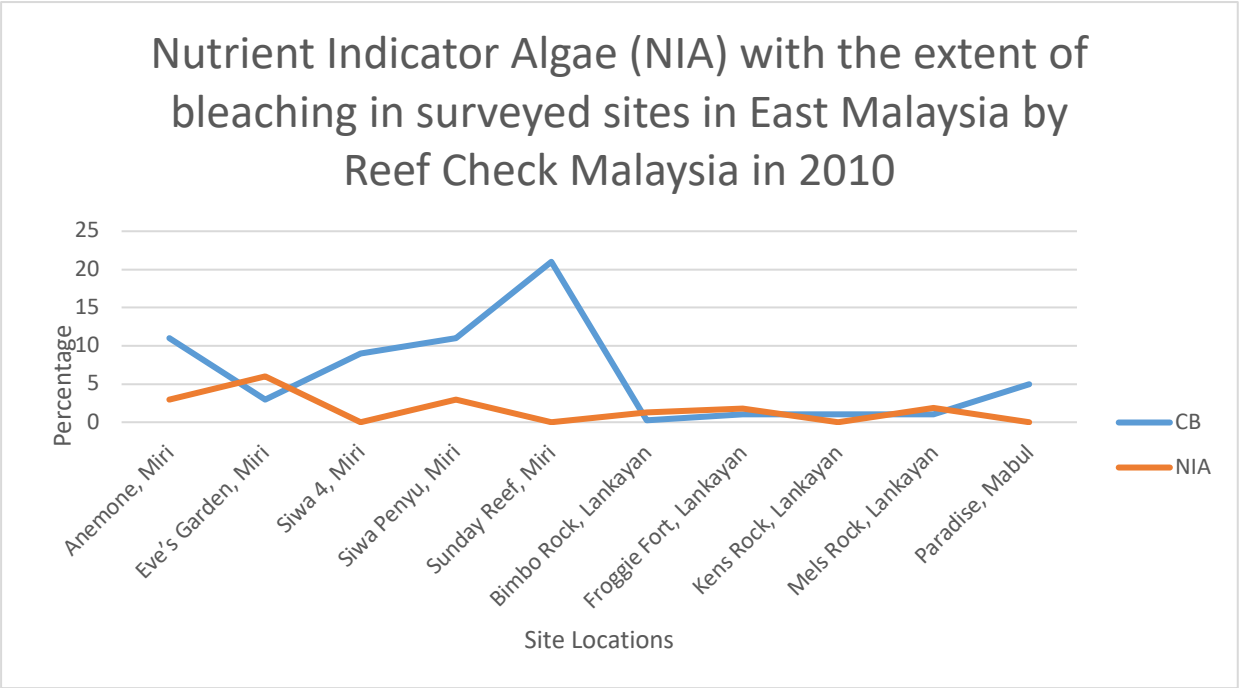


Figure 4.10: The trend of nutrient indicator algae with coral bleaching data which were recorded in surveyed sites in East Malaysia in 2010.

This Reef Check survey data was compared to environmental variables which are known to induce coral bleaching; Sea surface temperature (SST), Sea surface temperature anomalies (SSTA) and Degree Heating Week (DHW). Sea surface temperature anomalies (SSTA) in 2010 for Sabah and Sarawak (Figure 4.11) showed the CRW Sea surface temperature anomaly for Miri, Lankayan and Mabul Island. It was between 0-2°C in July 2010. Bleaching is connected to Thermal Stress Anomalies (TSAs) which are defined as areas where temperatures exceed by 1°C or more the climatologically warmest week of the year (NOAA, 2017).

Degree Heating Week 2010 was unclearly identified for Miri, Lankayan and Mabul Island during the July 2010 massive bleaching event (Figure 4.11) even though there was a bleaching event during the time period (from Reef check survey data). There was no report of coral mortality for East Malaysian reefs during this event.

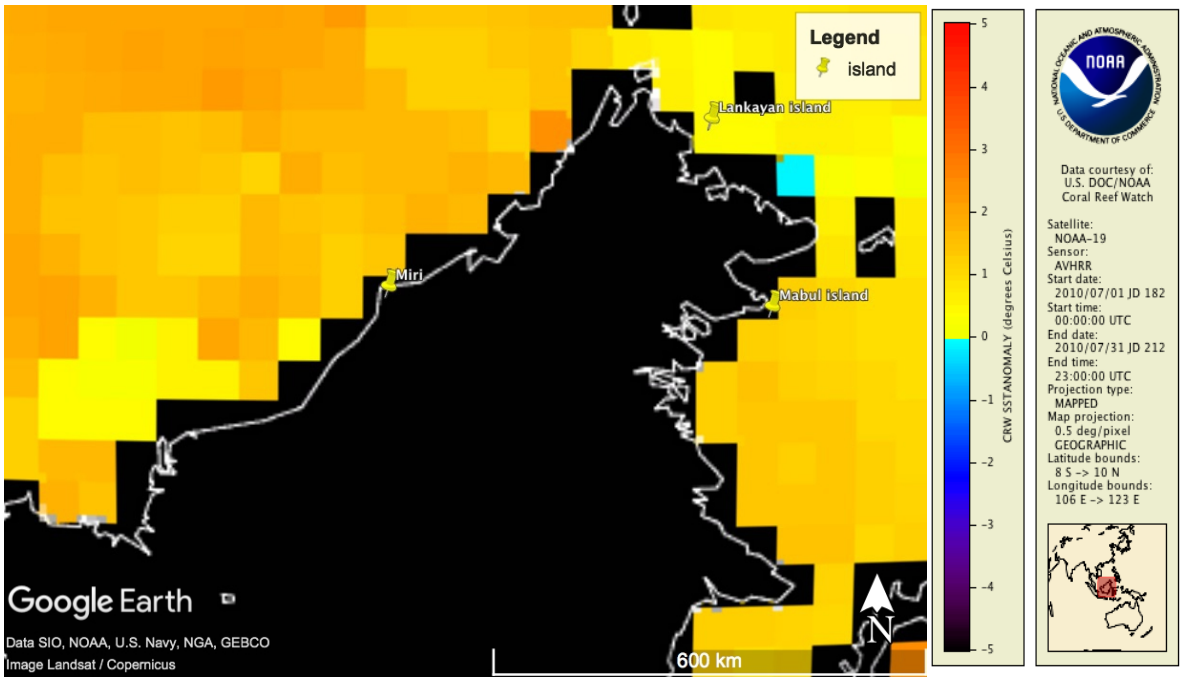


Figure 4.11: Sea surface temperature anomalies (SSTA) in 2010 for Sabah and Sarawak, East Malaysia, extracted from CoRTAD remote datasets and Google Earth to identify thermal stress. CRW Sea surface temperature anomaly for Miri, Lankayan and Mabul Island was between 0-2°C in July 2010. This NOAA Coral Reef Watch's monthly composite is derived from the corresponding data from all the twice-weekly periods that end in that month.

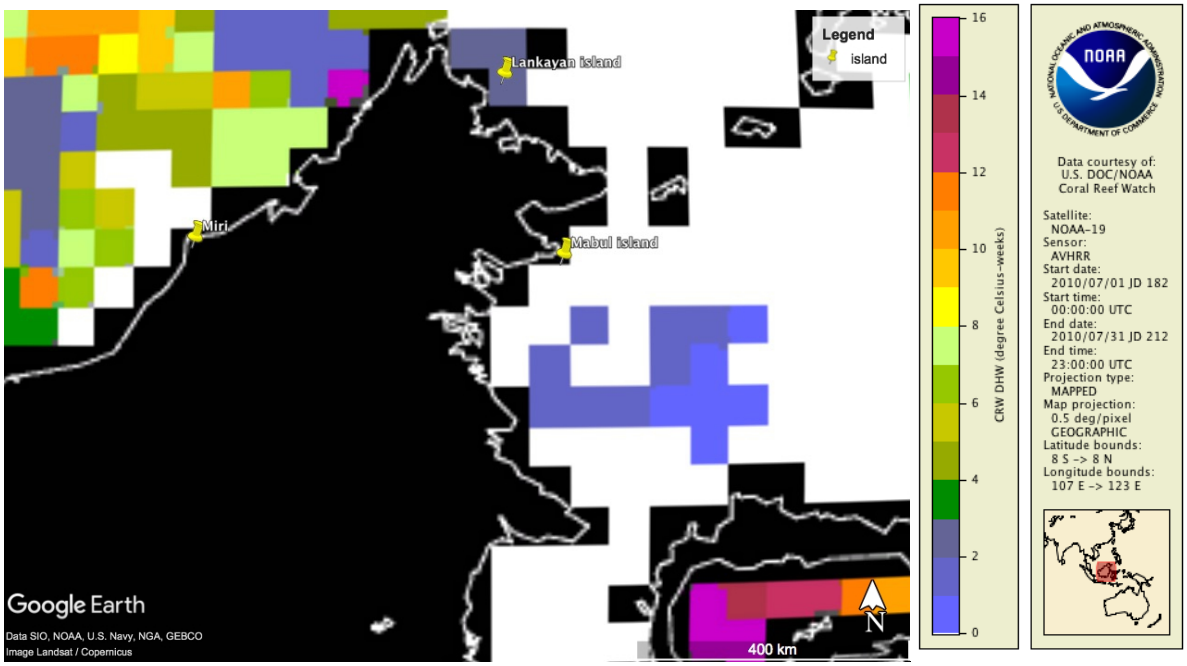


Figure 4.12: CRW Degree Heating Week for Miri, Lankayan and Mabul Island during the July 2010 massive bleaching event. This image is from NOAA Coral Reef Watch Annual Composite and Google Earth. Miri, Lankayan and Mabul Island were not clear identify the CRW-DHW in NOAA Coral Reef Watch's operational twice-weekly global 50-km satellite coral bleaching monitoring products.

The whole picture of DHW in Malaysia during the 2010 mass bleaching event (from May to September 2010) is shown in Figure 4.13. The coral bleaching DHW indicated that the SST in Malaysia started to increase in May 2010. DHW values of above 4°C-weeks exceeded the bleaching threshold temperature of 1°C from June to August 2010. In September 2010, SST had started to decline and later return to normal seawater temperature. In April 2010, none of the bleaching threshold temperatures were detected in the satellite image but later in May 2010, blue and green colour pixels can be seen in the Peninsular Malaysia map, with CRW DHW values of 0-2°C-weeks. In June 2010, the satellite image map shows CRW DHW values of >10°C-weeks, which indicated Bleaching Alert Level 2, bleaching and coral mortality would be likely to happen. This scenario continued until August 2010 but in September 2010, the NOAA satellite image showed only CRW DHW values of 0-2°C-weeks. Figure 4.14 shows the DHW annual composites for Malaysia from 2009 until 2011. Only in 2010 the DHW (Figure 4.14 b) exceeded the bleaching threshold temperature of 1°C, with values of 4°C-weeks.

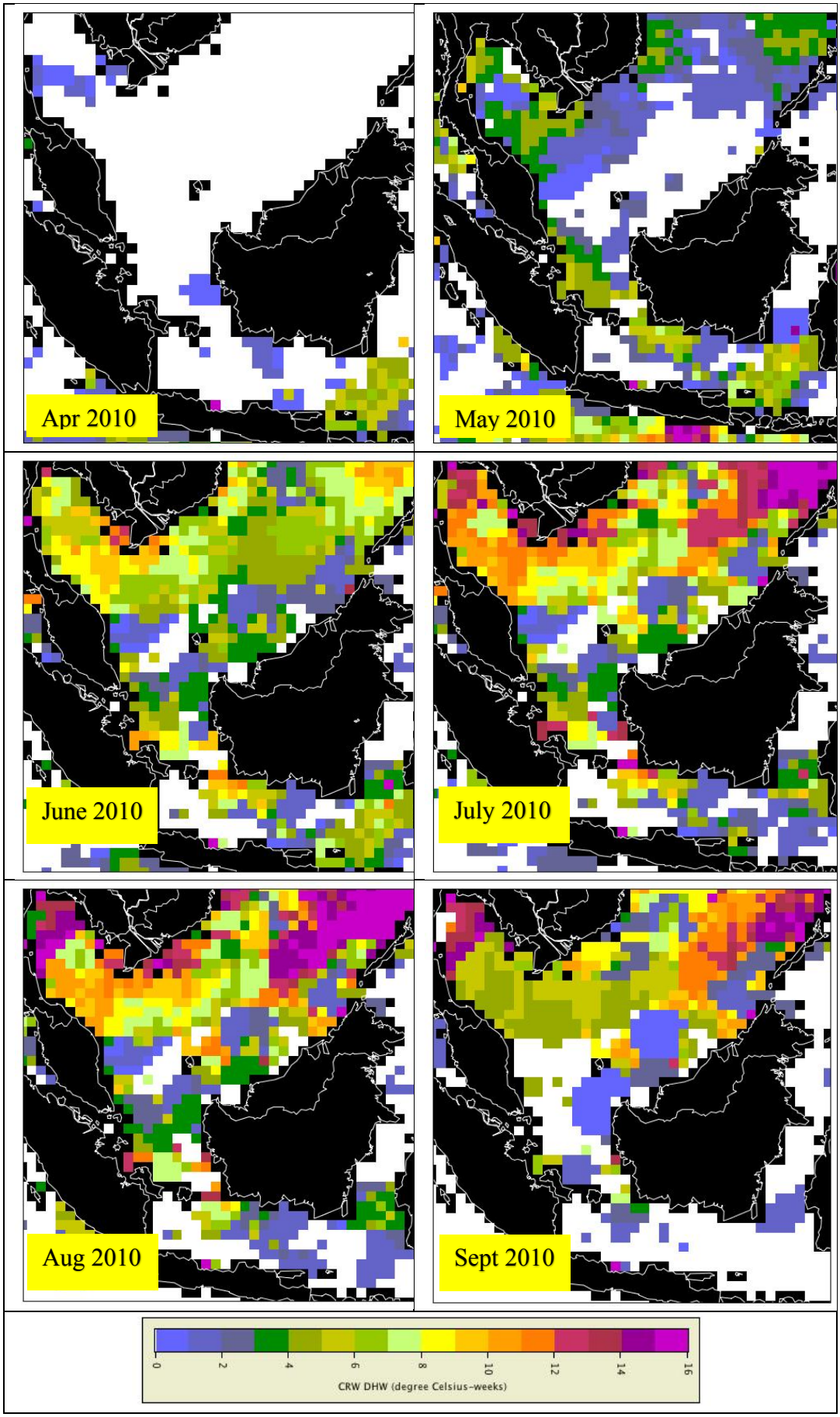
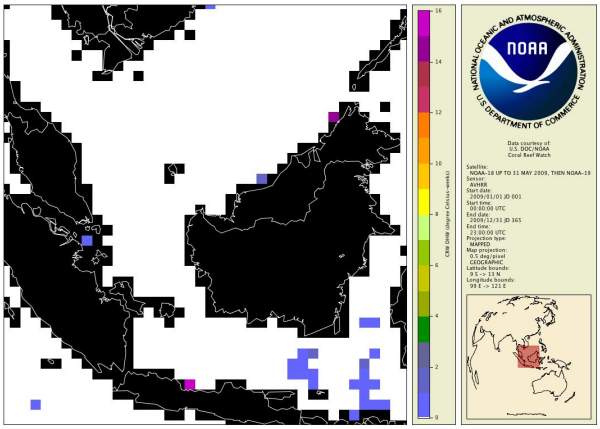
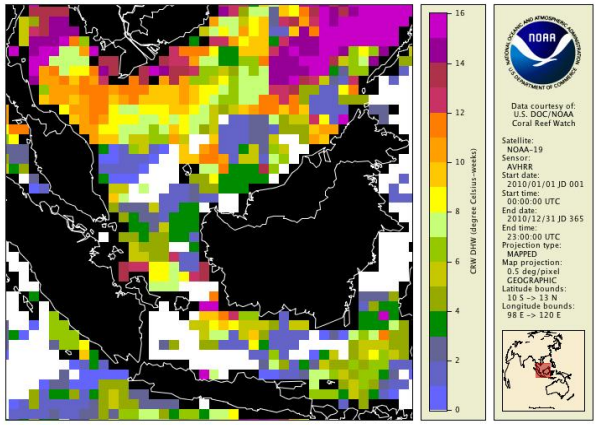


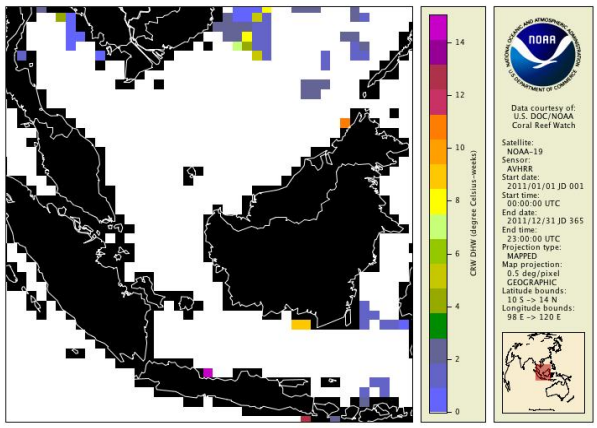
Figure 4.13: NOAA'S Coral reef watch coral bleaching Degree Heating Week (DHW) for Malaysia between April and September 2010. The colour scale is defined by CRW DHW values between 0 to 16°C-weeks.



(a)



(b)



(c)

Figure 4.14: NOAA Coral Reef Watch's operational twice-weekly global 50-km satellite coral bleaching monitoring products – Degree Heating Week (DHW) annual composite for Malaysia. From left to right; (a) DHW 2009, (b) DHW 2010 and (c) DHW 2011. This data is derived from the corresponding data from all the twice-weekly periods that end in that year.

4.3.3 Sea surface temperature during coral bleaching in Malaysia for 2009 and 2010

From the raw data on the coral bleaching surveys by Reef Check Malaysia in 2009 and 2010, the seawater temperature is shown in Figure 4.15 (East Malaysia survey data) for those sites where data is available. This data was similar to the NOAA Coral Reef Watch's operational twice-weekly global 50-km satellite coral bleaching monitoring – mean sea surface temperature for 2010 (Figure 4.16). This data shows that sea surface temperature of 29 to 31°C had caused coral bleaching. All the site locations recorded bleaching corals in 2009 and 2010 (refer to Table 4.5 and 4.6).

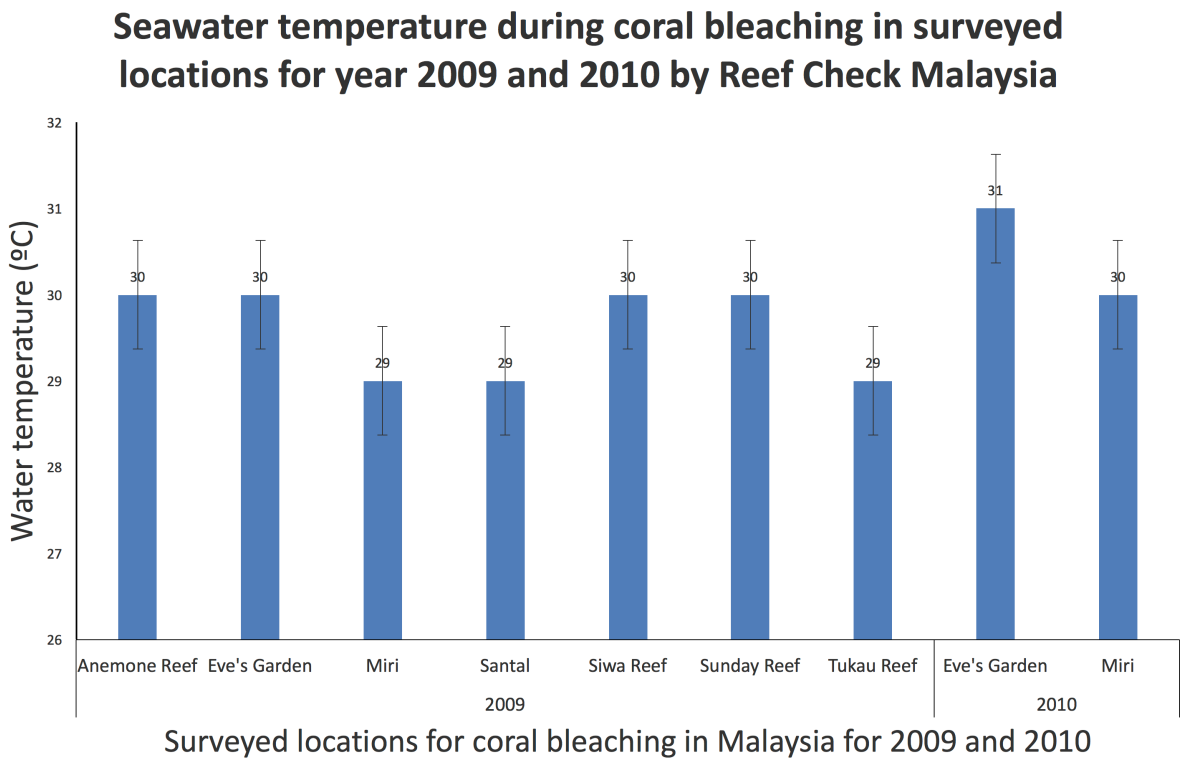


Figure 4.15: Survey by Reef Check Malaysia in 2009 and 2010 recorded coral bleaching events in East Malaysia. This table shows the seawater temperature which had caused bleaching at these location sites (Anemone Reef, Eve's Garden, Miri, Santal, Siwa Reef, Sunday Reef and Tukau Reef).

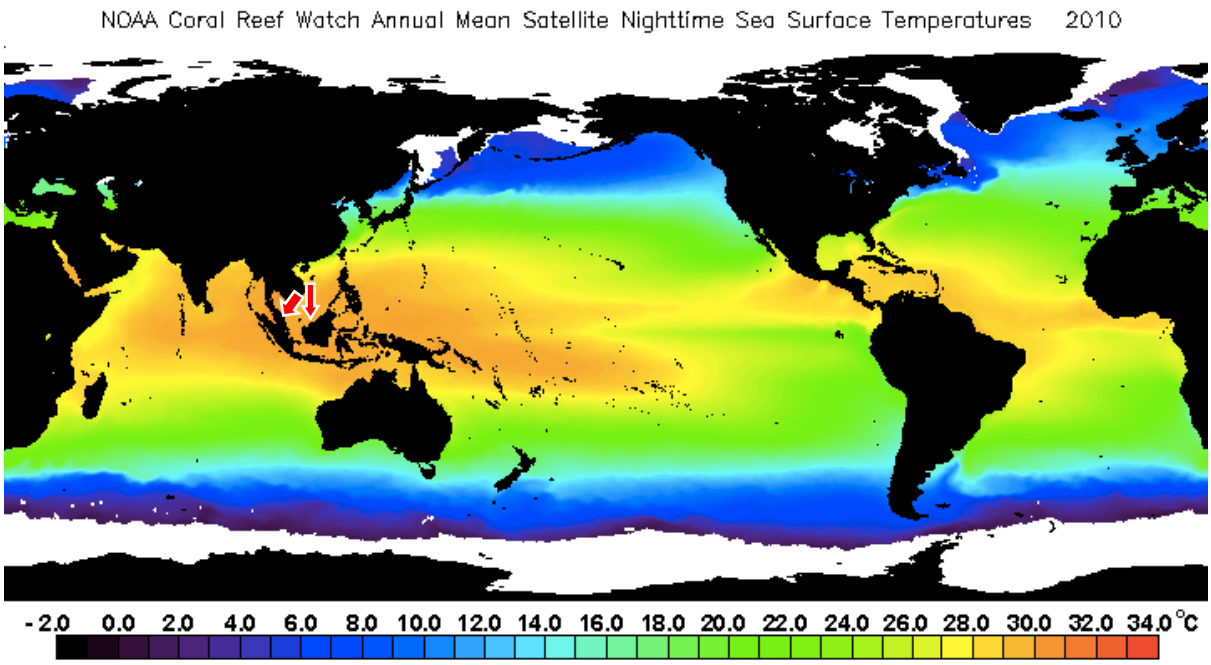


Figure 4.16: The NOAA Coral Reef Watch annual mean satellite nighttime sea surface temperature from NOAA's operational twice-weekly global 50-km satellite coral bleaching monitoring products for the year 2010. Sea surface temperatures around Malaysia (red arrow) area was in the range of 29-31°C.

4.3.4 Virtual stations in Mabul, Payar and Redang Island, Malaysia in 2010 and 2011

SST mean was analysed from Virtual Stations monitoring product, of Mabul Island, Payar Island and Redang Island data of year 2009 and 2010. From Reef check survey data, there were recorded coral bleaching in 2009 and 2010 throughout Malaysia. Using One-way ANOVA, SST mean is significant to induce bleaching only for Payar and Redang island but not Mabul island ($p>0.05$). During this seawater temperature, between 28-31°C, only Mabul and Redang island had recorded coral bleaching throughout 2009 and 2010. For Mabul island, the Figure 4.17 shows that between May until middle October 2010, alert 'bleaching watch' was recorded, with SST of 29°C. Mabul island recorded 5% of coral bleaching during this period (from Reef check

survey data). There was no Reef Check survey data for Payar island but from NOAA DHW (Figure 4.18) shows bleaching warning, alert level 1 and 2 in April until June 2010. For Redang island Virtual station (Figure 4.19), the 50-km SST is lower in January to April 2010 and 2011. The highest 50-km SST was observed remotely during those two years from May until July 2010, when the SST reached approximately 31°C. This was during the 2010 mass bleaching event. Later in August 2010, there was no further alert levels of bleaching.

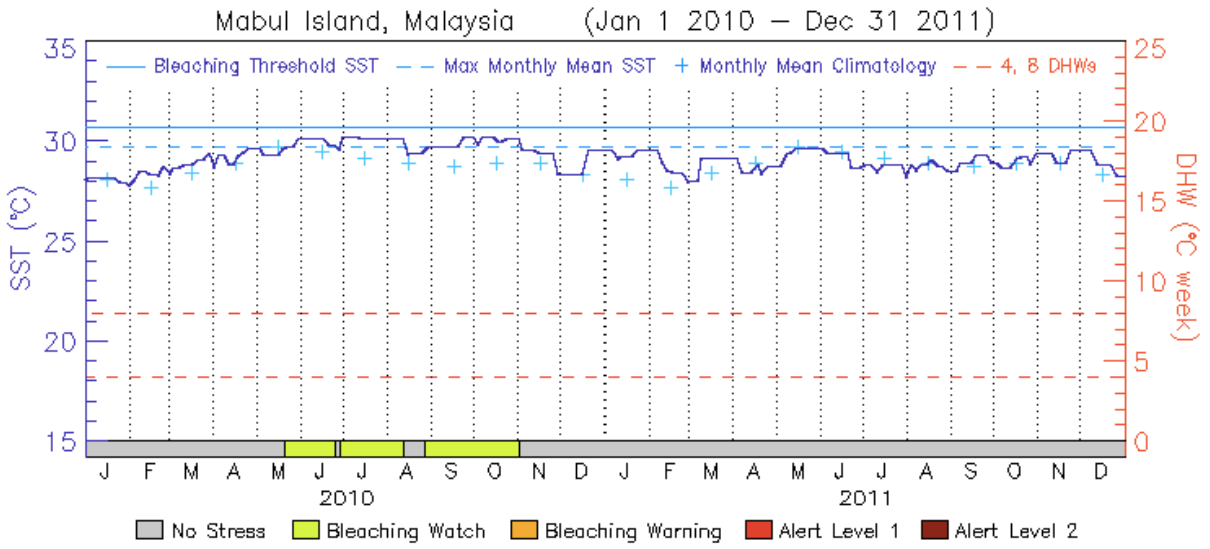


Figure 4.17: Virtual stations data for Mabul island between 2010 and 2011. The SST value is shown in the center graphs, and the vertical axis on the left. DHW is shown at the bottom of graphs and vertical axis on the right. Bleaching thermal stress is color coded at the most bottom of the graph box, categorized into five bleaching risk levels based on the Coral Bleaching Hotspot and Degree Heating Week values.

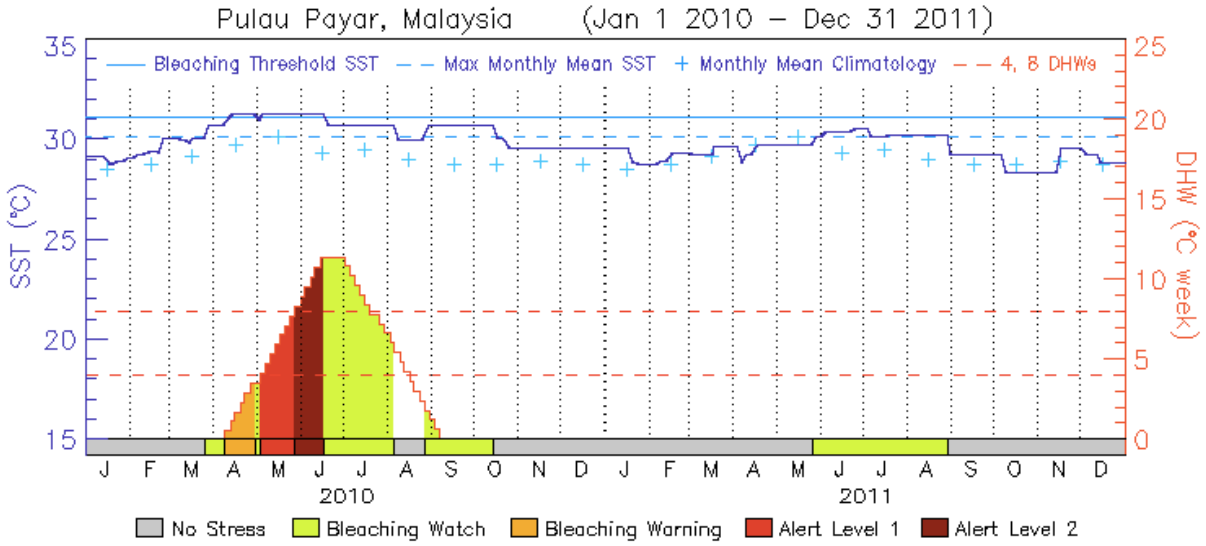


Figure 4.18: Virtual stations data for Payar island between 2010 and 2011

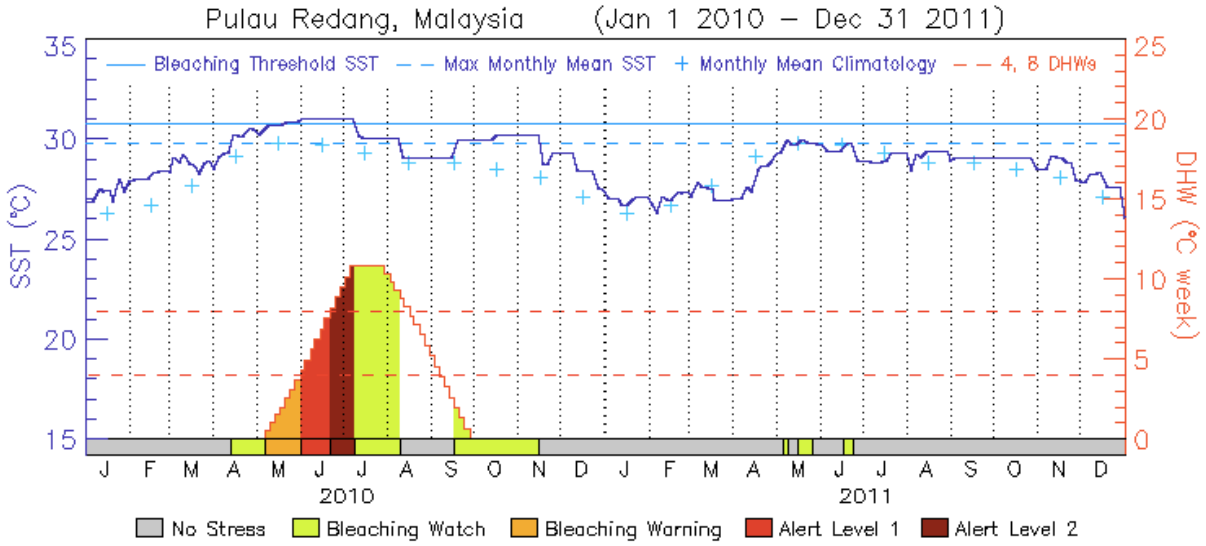


Figure 4.19: Virtual stations data for Redang island between 2010 and 2011.

4.4 Discussion

4.4.1 Reef Check survey data and NOAA monitoring products

From 2009 Reef Check data, high Recently Killed Coral (RKC) is possibly caused by the high tourism activities during the peak season each year since this island had been gazetted as a Marine Park in 1994 (Islam et al., 2013). Lighthhouse is a site location situated in Perhentian Kecil, an island near Perhentian island. Perhentian Kecil is a backpacker destination with small scale, locally owned chalets and a resort (Hamzah and Hampton, 2011) and Lighthouse is one of the famous snorkeling and diving area for the tourists. Tourist arrivals in Perhentian island was 90,000 in 2011, increased from 51,000 in 2004 (Department of Marine Park, 2012). Various activities by marine tourism such as diving and snorkeling can impact coral health (Saleh and Hassan, 2014). Poorly planned tourism development, ineffective sewage treatment and solid waste disposal, are some of the factors that affected the Perhentian's reef's health (Essays, 2013).

A report by Reef Check (2009) mentioned that Perhentian Kecil island had poor treated sewage problems from the chalets, which used septic tanks. The accommodation units have septic tanks that emptied their sewage sludge into the sea during the monsoon period (November to January every year) (Hamzah and Hampton, 2011). This might explain the highest Nutrient Indicator Algae (NIA) (23.8%) recorded in the area of Lighthouse, Perhentian Island in 2010. The presence of NIA is an indication of nutrient pollution in the water surrounding (Reefcheck, 2009). There was a study on the nitrogen and phosphorus nutrient along the coastal area in 2003, which showed that land development of the island contributed to the rising nutrient level in the coastal area of Perhentian (Chai, 2003). However, there was no water quality monitoring to clarify the nutrient pollution cause and source during the surveys of 2009 and 2010.

Tan (2013) reported that the excessive algae cover in 2011 in Perhentian island could not be kept naturally under control by algae-grazing fish. A study by the Malaysian Environmental Resources Management indicated that the sewage pollution in the tourist island was from eight resorts (out of 13) that have systems which require maintenance and desludging (Tan, 2013). From 2009 and 2010 surveys, high NIA was also reported in Redang island. The algae were recorded as growing on dead corals and rocks, which disturb the process of new coral recruitment (Tan, 2013). The Paku Besar site was nearby to the central tourist area on Redang Island, where eight resorts are located, and it might be the reason for the increased NIA in the area. Redang resorts have inefficient sewage systems and fertilisers were used for the resort's landscapings (ReefCheck, 2009). From the observation of the survey as well, there is a stream in the resort area that had bad odours related to sewage pollution (Tan, 2013). However, Ali et al. (2015), later concluded that the sewage contamination index clarified that Redang island was not contaminated by sewage (from surface sediment collected at 0-5km shoreline in 10 sampling stations around Redang island). Malaysia's national sewerage company had suggested a desludging operation is a better option than constructing a full-scale plant (limited water and electricity supply, lack of flat land, resorts are far separated) (Tan, 2013). While in Tioman island, coral degradation is probably due to run off from construction but generally the corals in Tioman rated as 'good', according to the Coral Reef Health Criteria (Reef Check, 2009).

In East Malaysia, Miri's reefs were reported to have been in poor condition since 2000, caused by river sedimentation (Pilcher and Cabanban, 2000). The studies also discussed that there were only 20-30% of live coral cover and the others were heavily covered with algae, indicative of nitrate enrichment in the area (Pilcher and Cabanban, 2000). Other than climate change, sedimentation, chemical pollution; indiscriminate fishing and ghost fishing nets also helped create the algae-dominated reefs in Miri islands (Borneo Post, 2010). The bleaching severity of

Miri's reefs presumably is caused by anthropogenic impact, worsened by the warming events in May to July 2010. Lankayan's reefs also had a few bleaching corals. In 2009, there was a Crown-of-thorns starfish (COTS) outbreak which resulted in high RKC cover in Lankayan (Reef Check, 2010). Corals in Lankayan were affected by the global warming and coral predation. Large scale of COTS removal programmes was conducted successfully but it must be continued to reduce numbers of this coral predator (Reef Check, 2010). While for Mabul island, there is no report of other causes of coral damage. Lankayan has no island communities and the waters are not a traditional fishing grounds (ReefCheck, 2011). In East Malaysia, the mass bleaching event effected *Porites*, *Montipora* and *Pachyseris* sp. but there was not much information on coral bleaching in 2009 and 2010 in Peninsular Malaysia.

The twice-weekly 50-km satellite coral bleaching DHW product shows the accumulated thermal stress which directly related to the timing and intensity of coral bleaching (NOAA, 2017). From this study, SST and DHW NOAA data are consistent with Reef Check survey data for certain areas in Malaysia, targeted locations by Reef Check Malaysia and also specific chosen Virtual Stations by NOAA. The bleaching impacts can be seen very localized, not a regionally wide bleaching event. The DHW NOAA in this study are only consistent with data bleaching of Tioman, Redang island of Peninsular Malaysia and Miri island in East Malaysia. There is also documentation providing bleaching data for Tioman (Guest et al., 2012) and Miri (Tan, 2013). Tioman and Redang is located in East Coast Peninsular Malaysia, and Miri island is in the middle part of East Malaysia.

The 2010 mass bleaching event can also be related to NOAA Coral Reef Watch Virtual Stations which contains SST and DHW combined data from January 2010 to December 2011 in Mabul, Payar and Redang Island. The virtual stations in Malaysia were only situated in these three

islands. Mabul island is situated in East Malaysia, Payar island in West coast of Peninsular Malaysia and Redang island is in East coast of Peninsular Malaysia. These three islands are relevant to this study because they represent Malaysian waters. Personal communication with Chelliah (2012), indicated that there was no bleaching event recorded by Reef Check surveys in 2011, which correlad with the NOAA Coral Reef Watch Virtual Stations data from early 2010 to end of 2011.

Surveys in 2010 only reported bleaching events in Tioman, Redang and Perhentian islands. 50% of the corals in these three major islands were bleached, with two-thirds of those colonies were completely white (Tan and Heron, 2011). From Reef Check survey data and other documentations (Guest et al., 2012; Praveena et al., 2012; Tan, 2013), it appears that not all of the Malaysian bleaching data from surveys and NOAA monitoring products are related to the warming event. Reef Check (2010) reported that bleaching of corals in Redang island in 2009 was caused by white band disease, eventhough there were DHW values of bleaching and mortality in the satellite images. In contrast, the values of the DHW observed in 2010 are considered sufficient to cause bleaching-induced mortality for Tioman island. 25 weeks after the mass bleaching event (April 2010) in Tioman island, Guest et al., (2012) reported coral mortality in the specific area (Table 4.4). While for Miri island, even though there are bleaching records by Reef Check surveys, the DHW values in 2009 and 2010 did not reflect a clear view of bleaching in the exact location (by using longitude and latitude). As discussed earlier, Miri's reefs (Sarawak) were impacted by anthropogenic impact (fishing, pollution, algae-dominated reefs) earlier before the mass bleaching event. The other state in East Malaysia is Sabah, and 80% of their coral reefs were damaged by overfishing and fish blasting scenarios (Yasin et al., 1998). Islands in Sabah are far from land and out of the normal renage of enforcement operations, caused them to be among the worst reefs in Malaysia (Praveena et al., 2012). While for Redang

reefs, they were exposed to sewage pollution from resorts since before 2009. Tourism impact was reported to be the major cause of coral reef degradation in Perhentian, Redang and Tioman islands since 2008 (Chai, 2009). These reefs were not healthy enough during the global warming catastrophe, and frequent short-term stress (1998, 2009 and 2010) with the continuous human impact may lead to corals degradation for Malaysia in a long run.

4.4.2 Coral reef management in Malaysia

Higher sea temperatures and coral bleaching is a major threat to coral reef health, especially on stressed corals impacted by the human threats in its environment. NOAA's Coral Reef Watch monitoring products publish satellite data on the coral bleaching indication and email the alerts for a quick management strategy responses for all the bleaching areas. On the country scale, continuous monitoring by the researchers and stakeholders should take place and actions plans are required to respond to bleaching events. In Malaysia, the Coral Reef Bleaching Responsce Commmittee (CRBRC) in the Department of Marine Parks Malaysia (DMPM) have published a Malaysia Coral Reef Bleaching Response Plan as a guide for monitoring and managing future mass bleaching events with the stakeholders.

There are gaps in coral reef studies in Malaysia which can be solved if there are continuous collaborations between local and international researchers and research by local universities (Praveena et al., 2012). Coordination of coral reef surveys by the institutions (government, academia and NGOs) are required to (1) apply a standardized method because when there are different surveys, the data is difficult to compare and analyse, (2) the data should be distributed between involved institutions to create a proper coral database (Reef Check, 2011).

Local threats on Malaysian reefs must be managed by community involvement, so that survivability coral reefs can be built to withstand the future bleaching events (Reef Check, 2011). Coral reefs in Malaysia are protected under (1) Environmental Quality Act 1974, (2) Fisheries Act 1985, (3) Pesticides Act 1974, (4) Plant Quarantine Act 1976 and Customs (Prohibition of Exports Amendment No. 4 Order 1993 (Praveena et al., 2012). Department of Marine Park Malaysia, Department of Fisheries, local universities and international agencies have been working on conducting regular monitoring at Marine Parks in Peninsular Malaysia, Sabah Parks in Sabah's marine parks and Sarawak Forestry Department together with Fisheries Department in Sarawak. However, there is poor coordination and compilation of data from those authorities resulting in no data centralization for data analysis or archiving for coral reefs in Malaysia (Praveena et al., 2012). There are still gaps to be filled in the qualitative, quantitative and biogeographical data on coral reefs communitites in Malaysia (Praveena et al., 2012). Studies to understand effects of MPAs management on coral reefs health and impact on pollution on coral reefs growth are needed. The Department of Marine Parks Malaysia is recommended to recognize the threat posed by mass coral bleaching and take those actions which are applied in well-established MPAs; (1) implement management plans to respond to mass coral bleaching, and (2) build long term resilience (Reef Check, 2010). On the other hand, there must be a well established legal system to reduce pollution, illegal and destructive fishing activities (Praveena et al., 2012). But, according to Saad et al. (2012), marine water-quality monitoring near islands is outsourced to the private sector and there are no published guidelines on quality assurance for sampling, sample transportation, documentation or laboratory analysis.

4.4.3 Thermal stress and Nutrification for Coral Susceptibility

Human activities in the coral reef ecosystem resulted in an elevated input of nutrients in the waters which affected the corals (D'Angelo and Wiedenmann, 2014). They discussed that nitrate enrichment combined with heat and light stress water conditions might make the coral more bleaching. It is said that elevated temperature is known to be the cause of mass coral bleaching events, but enrichment inorganic nitrogen can give negative effects of thermal stress as well (Serrano et al., 2017). A study by Fabricius et al. (2013) showed that nitrate alone had no measurable effect on survival, bleaching and recovery in coral species. They found that nitrate enrichment can worsen thermal stress on coral communities.

Earlier recorded in Naim (1993), hard corals which exposed to nutrient-enriched submarine groundwater dishcharge, already dominated of algae were found bleached and died during the hot season. The bleaching was said to be not caused by the increased temperature, but the overgrowth of corals with algae had caused a physiological stress onto corals. *Acropora millepora* and *Montipora tuberculosa* went through reductions in maximum quantum yields, lower survival and slower recovery after heat stress and nitrate-enriched treatments for 90 days (Fabricius, 2013). A study by Miller (2013) found that *Porites cylindrical* in nutrient-enriched waters showed rapid loss of symbiotic algae after exposed to heat stress, compared to control samples. Wooldridge and Done (2009) also found that the combined effects of nutrient enrichment and thermal stress give negative effect on coral's health.

For this study, there is shown no data nutrient enriched waters caused the coral bleaching. the data did not give any significant relationship between nutrient enrichment and coral bleaching. reefcheck (2017) mentioned that after 2010 mass bleaching event, there was a substantial

increasing data of coral recovery in 2011 and 2012. There is a report in Perhentian Island where local impacts have been affecting the corals after bleaching event which worsen the corals (Reefcheck, 2017).

As conclusion, there is need on managing strategies to improve water quality, so that it can enhance the reef resilience, thus improve the thermal tolerance of coral species. From the management perspective, it is recommended for them to manage number of tourists to reduce physical damaged on most visited sites (Reefcheck, 2017). They said the corals that were threatened by anthropogenic impacts can recover easily when bleaching stressors are removed.

4.5 Conclusion

Before the 2010 mass coral bleaching event in Malaysia, Malaysian reefs had gone through 1997/98 global event but it was a mild bleaching event there and occurred only in certain areas. But in 2010, the coral bleaching was severe and widespread causing the government to take action to shut down the islands from any activities. Malaysia had to undertake an effective management strategy to protect the reefs from anthropogenic threats, so that the corals were healthy enough to survive the warming event. Coral Reef Watch (CRW), a program under NOAA Coral Reef Conservation Program, has been operating the coral bleaching thermal stress monitoring using satellites for the coral reef communities worldwide. CRW's operational satellite data products such as Coral Bleaching Hotspots and Degree Heating Weeks, SST measurements, bleaching alert area, Virtual Stations and Satellite Bleaching Alert email system; provide the reef managers, researchers and stakeholders with important information so that they can understand, predict and monitor the development of mass coral bleaching event (Liu et al., 2013). Researchers and managers can use these products by targeting *in situ* surveys, collecting

and manage bleaching data for before, during and after mass coral bleaching events. Predicting coral bleaching by satellite data, routine coral reef monitoring by the Malaysian coral reefs management and bleaching action plan response by the managers and governments; are necessary to improve the reef environment resilience from human threats and climate change.

References

Ali M.M., Musa K.A., Chiu T.R., Ali S.A.M. and Humrawali N. 2015. Sterols in surface sediments of the Redang Island, Terengganu. *Malaysian Applied Biology* **44: 1**. pp. 41-49.

Ang, P., 2010. Status of coral reefs in East Asian seas region. *Japan Wildlife Research Centre*, pp.65-77.

Chelliah A.J., Yee C.S., Amri A.Y., Abd Adzis K.A., and Hyde J. 2014. Status of reefs in selected Southeast Asia Countries. Reef Check Malaysia Bhd.

Chai W.K. 2003. The effect of land development towards the distribution of nitrogen and phosphorus nutrient along the coastal area of Perhentian island, South China Sea. Undergraduate thesis, Bachelor of Science in Marine Science, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi (KUSTEM). 109p.

Chai N.W. 2009. Ecotourism and environmental conservation in small islands in the East Coast of Peninsular Malaysia. *Malay J Environ Manage* **10:**53–69

Chelliah A. 2012. Personal communication.

Department of Marine Park. 2010. Available at http://www.dmpm.nre.gov.my/ [Accessed 20th August 2017].

Summary report of MPAs Malaysia. Available at http://www.reefbase.org/key_topics/pdf/ Malaysia%20mpa.pdf [Acessed 28th August 2017].

Fabricius, K.E., Cséke, S., Humphrey, C. and De'ath, G., 2013. Does trophic status enhance or reduce the thermal tolerance of scleractinian corals? A review, experiment and conceptual framework. *PloS one* ***8*** (1): p.e 54399.

Fisher J., Nawaz R., Fauz, R., Nawaz F., Said Md Sadek, E., Abd Latif Z. and Blackett M. 2008. Balancing water, religion and tourism on Redang Island, Malaysia. Environment Resource Letter **3 (2):** pp. 024005.

Hamzah, Amran and Hampton, Mark P. 2012. Tourism Development and Non-linear Change in Small Islands: Lessons from Perhentian Kecil, Malaysia. Working paper. University of Kent. (Unpublished)

Idris, M.H., Muta, H.Z. and Arshad, A., 2006. Status of coral reefs species at patricia shoals, Bintulu, Sarawak, Malaysia. *J App Sci Res* **10** pp.816-820.

Islam, G.M.N., Noh, K.M., Yew, T.S. and Noh, A.F.M., 2013. Assessing environmental damage to marine protected area: a case of Perhentian Marine Park in Malaysia. *Journal of Agricultural Science*, ***5*** **(**8): p.132.

Liu G., Rauenzahn J.L., Heron S.F., Eakin C.M., Skirving W., Christensen T.R.L., Strong A.E. and Li J. 2013. NOAA coral reef watch 50 km satellite sea surface temperature-based decision support system for coral bleaching management. *NOAA Technical Report NESDIS* **413**. National Oceanic and Atmospheric Administration. Washington DC.

Miri nature society: coral reefs in Miri under threat. 2010. *Borneo Post online.* June 21, 2010.

Naim, O., 1993. Seasonal responses of a fringing reef community to eutrophication (Reunion Island, Western Indian Ocean). *Marine Ecology Progress Series*, *99*, pp.137-151.

NOAA Coral Reef Watch. 2011. NOAA Coral Reef Watch 50-km Satellite Monitoring Products. Silver Spring, Maryland, USA. Accessed http://coralreefwatch.noaa.gov/satellite/vs/index.php.

NOAA Coral Reef Watch. 2013. NOAA Coral Reef Watch 50-km Satellite Monitoring Products. Silver Spring, Maryland, USA. Accessed http://coralreefwatch.noaa.gov/satellite/vs/index.php.

NOAA Coral Reef Watch. 2017. NOAA Coral Reef Watch 50-km Satellite Monitoring Products. Silver Spring, Maryland, USA. Accessed http://coralreefwatch.noaa.gov/satellite/vs/index.php.

Praveena, S.M., Siraj, S.S. and Aris, A.Z., 2012. Coral reefs studies and threats in Malaysia: a mini review. *Reviews in Environmental Science and Bio/Technology* **11(1):** 27-39.

Pilcher, N. and A. Cabanban. 2000. The Status of Coral Reefs in Eastern Malaysia. Global Coral Reef Monitoringlan Network (GCRMN) Report. Australia Institute of Marine Science, Townsville.

Reef Check. 2009. Annual Survey Report. Reef Check Malaysia Bhd. Kuala Lumpur.

Reef Check. 2010. Annual Survey Report. Reef Check Malaysia Bhd. Kuala Lumpur.

Reef Check. 2011. Annual Survey Report. Reef Check Malaysia Bhd. Kuala Lumpur.

Reef Check. 2013. Annual Survey Report. Reef Check Malaysia Bhd. Kuala Lumpur.

Reef Check. 2015. Annual Survey Report. Reef Check Malaysia Bhd. Kuala Lumpur.

Saad, J., K. Hiew, and N. Gopinath. 2012. Review of Malaysian Laws and Policies in Relation to the Implementation of Ecosystem Approach to Fisheries Management in Malaysia. Publication. Honolulu, Hawaii: The USAID Coral Triangle Support Partnership. Print.

Saleh, M.S.M. and Hasan, N.N.N., 2014. Ecotourism and Coral Reef: Analysis of Coral Reef Conservation Awareness among Stakeholders in Perhentian Island, Malaysia. *American Journal of Tourism Management*, *3*(1A), pp.7-12.

Serrano, X. M., Miller, M. W., Hendee, J. C., Jensen, B. A., Gapayao, J. Z., Pasparakis, C., Baker, A. C. (Accepted/In press). Effects of thermal stress and nitrate enrichment on the larval performance of two Caribbean reef corals. *Coral Reefs*, 1-10. DOI: 10.1007/s00338-017-1645-y.

Tan C.H. 2013, December 13. Pulau Perhentian reefs under attack from algae. *The Star Online.* Retrieved from www.thestar.com.my.

Tun K. et al. 2010. A regional overview on the 2010 coral bleaching event in Southeast Asia in *Global Coral Reef Monitoring Network: Status of coral reefs in Southeast Asia 2010*. Publisher Ministy of the Environment, Japan.

UK Essays. November 2013. Environment of Perhentian Island Malaysia Tourism Essay. [online]. Available from: https://www.ukessays.com/essays/tourism/environment-of-perhentian-island-malaysia-tourism-essay.php?cref=1 [Accessed 10 August 2017].

Chapter 5

The Effect of Temperature and Irradiance on Photosynthetic Quantum Yield of Three Scleractinian Corals

5.1 Introduction

Scleractinian corals contain a certain genus of symbiotic dinoflagellate algae, *Symbiodinium* sp., which helps to provide the host with respiration gases and nutrients. When the zooxanthellae are expelled from the corals, the coral symbiosis will effectively lose photosynthetic pigments, and this will disrupt the symbiosis resulting in coral bleaching (Hill and Ralph, 2006). Global warming is a significant threat to the future of coral reef ecosystems because coral bleaching is related to elevated sea temperatures (Guest et al., 2012). Mass coral bleaching is caused by sea surface temperature (SST) and has resulted in significant losses of corals in many parts of the world (Hoegh-Guldberg, 1999). It is also caused by synergistic effects such as high solar irradiation due to clear skies and periods of calm weather (Hoegh-Guldberg, 1999). The U.S. National Oceanic and Atmospheric Administration (NOAA) Coral Reef Watch program has developed a daily global 5-km product based on satellite observations to monitor thermal stress on coral reefs (Liu et al., 2014). Significant coral bleaching may occur when Degree Heating Week (DHW) values reach 4°C-weeks. If the DHW values reach 8°C-weeks, bleaching is widespread and significant mortality will be expected (NOAA, 2017). When temperatures exceed 1°C above the highest monthly mean temperature, it can cause stress to corals (Glynn and D'Croz, 1990).

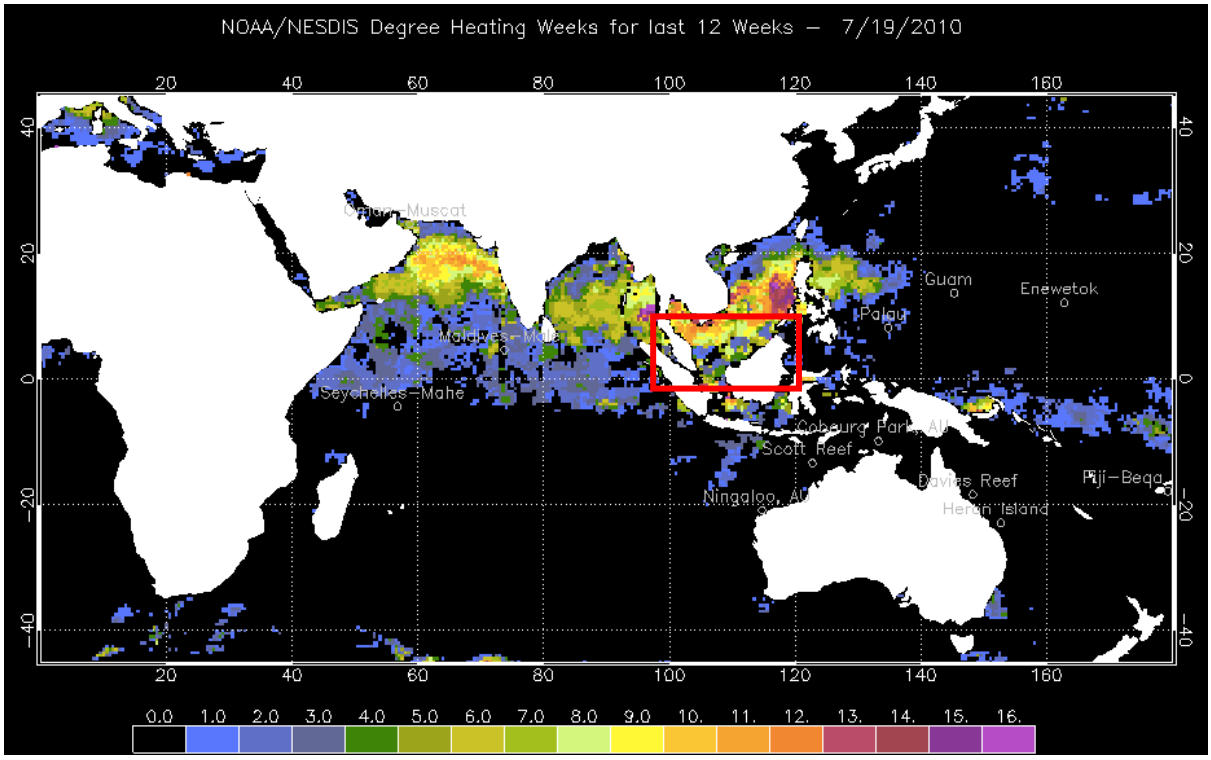


Figure 5.1: Degree Heating Weeks (DHW) in Malaysia (Red box) during the mass bleaching events in July 2010 (NOAA, 2017)

Hoegh-Guldberg and Jones (1999) found that raised sea temperature of 1-2˚C and high solar irradiance can cause coral bleaching resulting in mortality. Thermal-stressed bleaching is known as the major cause for the loss of many of coral reefs (Smith et al., 2005) throughout the world but the thermal tolerance and bleaching susceptibility of each coral is not the same, and can vary greatly even within species (Borell, 2008). Based on Guest et al (2012), the severity of coral bleaching is different in space and time and depends on the thermal stress magnitude (Kleypas et al. 2008), irradiance levels (Hoegh-Guldberg, 1999; Winters et al., 2003), symbiont types (Berkelmans and van Oppen, 2006) the species composition of the coral assemblage (Loya et al., 2001; Marshall and Baird, 2000), and the thermal history of the site (Thompson and van Woesik, 2009).

Most coral bleaching studies focus on the algal symbiont because of their differential species-specific sensitivity to temperature in each coral species (Strychar and Sammarco, 2009), and species composition is one of the strongest drivers of this variation due to a predictable hierarchy of coral taxa susceptibility (Loya et al 2001). Hardier, slow-growing massive corals will replace less hardy, fast growing branching coral species on reefs in the future (Loya et al., 2001 and Hughes et al., 2003). *Acropora* and *Pocillopora* succumb to small increases in SSTs compared to *Porites* spp. (the massive taxa) and some Faviids take longer to bleach because they are less sensitive (Baird and Marshall, 2002; Strychar and Sammarco, 2009). If the coral survives, the bleached corals may experience the re-establishment of the symbiont-host relationship (Strychar and Sammarco, 2009). This suggests that coral hosts are more resistant to a rising temperature than their own zooxanthellae (Strychar and Sammarco, 2009). Thermal stress is also related to coral disease outbreaks, where warm temperatures anomalies were significant with the frequency of white syndrome disease across 48 reefs on the Great Barrier Reef, Australia, based on the annual surveys of 6 years (Bruno et al., 2007). The frequency of warm anomalies of 1°C or higher can cause physiological stress in the coral host, which can lead to coral disease outbreaks (Bruno et al., 2007).

Bleaching is caused by photoinhibition of photosynthetic electron transport and the consequent photodamage to PSII and the production of damaging reactive oxygen species (ROS) in the zooxanthellae (Smith et al., 2005). Lesser (1996) noted that either exposure of cultured zooxanthellae towards sublethal temperature perturbations alone or UV radiation alone can cause photoinhibition. Photoinhibition is defined as the light-dependent reduction of photosynthetic capacity and/or photosynthetic efficiency (Bhagooli and Hidaka, 2003). Bhagooli and Hidaka (2003) also suggested that high temperature alone can damage the PSII, on its reaction center protein D1. Most studies concluded that corals will have low maximum

quantum yield of the PSII based on the ratio of variable F_v to maximal F_m fluorescence (F_v/F_m) during a bleaching episode (Falkowski et al., 1994; Bhagooli and Hidaka, 2004). Gorbunov et al., (2001) explained two types of photoinhibition, the dynamic and chronic, where dynamic photoinhibition is rapidly reversible within hours but chronic photoinhibition demonstrates being either reversible within days, or not reversible at all (Bhagooli and Hidaka, 2003), and mostly happens during high irradiance and temperature (Bhagooli and Hidaka, 2004). Ferrier-Pagès et al (2007) concluded that solar radiation (photosynthetically active radiation, PAR and ultraviolet radiation, UVR 290-400nm) can cause stress to corals. Among the earliest reports on the effect of ultraviolet radiation causing bleaching is that by Gleason and Wellington (1993), which reported the mass bleaching events of 1987 and 1990 in the Caribbean were due to ultraviolet radiation of 280-400 nm band. Hoegh-Guldberg and Smith (1989) proved that 7-h light stress (full sunlight and temperature > 30°C), reduced the amount of photosynthetic pigments, not the number of zooxanthellae per surface area of coral *S. pistillata* and *S. hystrix.* Most studies (Lesser et al., 1990; Lesser, 1997) reported an involvement of solar UV radiation with the production of toxic reactive oxygen species (ROS) which will damage cellular proteins and photosynthetic membranes (Ferrier-Pagès et al., 2007).

Ainsworth et al. (2008) reported that when temperatures exceeded 32°C for a continuous period, there are decreased values of dark-adapted yield (<0.3) and cell density reduction occurred (50% reduction), causing *Acropora aspera* to bleach. This study used an Imaging-PAM fluorometer (Walz, Germany) to avoid handling effects on tissues and cell responses (Ainsworth et al., 2008). In Takahashi, et al. (2004), *Acropora digitifera* showed reductions in F_v/F_m at light intensities higher than 250 µmol quanta $m^{-2}s^{-1}$ at a temperature of 28°C, and at temperature of 32°C but light intensities of only 100 µmol quanta $m^{-2}s^{-1}$. These experiments suggested that heat stress enhances the susceptibility of the photosynthetic system to photoinhibit (Takahashi et al., 2004).

The Diving- PAM (Waltz, Effeltrich, Germany) was used in the study to measure the chlorophyll measurements after incubating the stressed corals with a 10-min dark-adaptation (Takahashi et al., 2004). Takahashi et al. (2013) demonstrated that each of six *Symbiodinium* spp. had their own thermal acclimation mechanism where the F_v/F_m values declined at different increased temperatures between 34°C and 36°C. In another experiment, F_v/F_m was measured using a PAM-2000 chlorophyll fluorometer (Heinz, Walz) after 10-min dark-adaptation (Takahashi et al., 2013). While F_v/F_m were measured for different genotype clade compositions of zooxanthellae in *Pavona divaricata* and *P. decussate* in Okinawa, Japan by using a Mini-PAM fluorometer (Walz) (Suwa et al., 2008). There were decreases in F_v/F_m values in *P. divaricata* and *P. decussata* when harbouring clade C zooxanthellae during cold and warm seasons, while clade D zooxanthellae in *P. divaricata* were not affected by the seasonal fluctuations (Suwa et al., 2008). The lowest maximum quantum yield measurements were recorded in the two species after 3-h stress treatments of low temperature (18°C) + high light (1000 μmol photons m^{-2} s^{-1}) and high temperature (33°C) and high light treatments (Suwa et al., 2008). A decline in dark-adapted F_v/F_m (15min dark adaptation in this study) generally reflects that photoinhibition has occurred during the experiments (Jones and Hoegh-Guldberg, 2001; Bhagooli and Hidaka, 2003). Winters et al. (2003) measured effective quantum yield ($\Delta F/F_m'$) under natural light for colonies of coral *S. pistillata in situ* (shallow 2 m and deeper 11 m corals) using a Diving-PAM (Walz). Effective quantum yield of PSII measured during night time is equivalent to maximum quantum yield, F_v/F_m.). Slow fluorescent measurements (PAM) and fast-induction kinetics (PEA) were performed on *Pocillopora damicornis* using a Diving-PAM (Walz, Germany) and Plant Efficiency Analysis (PEA) respectively (Ulstrup et al., 2005). The samples went through dark-acclimatization for 10 minutes which was sufficient to full dark-adapt the samples (Ulstrup et al., 2005). The zooxanthellae of *P. damicornis* decreased values of F_v/F_m significantly for slow fluorescent measurements when exposed to anaerobic conditions (40 ± 5% air content with a

flow regime of ca. 2 cm s^{-1}, 0 to 2% air content with a flow regime of approximately 2 cm s^{-1}, and 0 % air content with no flow) (Ulstrup et al., 2005). *Pocillopora damicornis* in Tioman Island, Malaysia was chosen to study their effective quantum yield (light-adapted) ($\Delta F/F_m'$) *in situ* for 21 days and maximum quantum yield (dark-adapted) were also taken (F_v/F_m) at dawn between 06:00-07:00 by using a Diving-PAM (Walz, Germany) (Abdul-Adzis et al., 2009). This is the first data of baseline standard of *P. damicornis* yield value of normal state for the east coast of Peninsular Malaysia which is 0.68-0.72 (Adzis et al., 2009). Later in 2012, effective quantum yield (F/F_m') was also being studied to evaluate the severity of the massive coral bleaching event for *Acropora formosa* and *P. damicornis* in Tioman Island, Malaysia by using a Diving-PAM fluorometer as well (Adzis et al., 2012). And according to this, there are no publications of the changes of photosynthetic capacity changes of coral reef bleaching in Malaysia. In 2013, there was one more study on the effective quantum yield but on another marine organism, the sea anemone *Heteractis magnifica* in Tioman Island, measured by Diving-PAM (Walz, Germany) for diurnal patterns (Khoo and Mazlan, 2013). The *in-situ* measurements were meant to provide baseline data of anemone's daily yield values (0.74±0.11) (Khoo and Mazlan, 2013).

It is clear that there are no baseline standard data for maximum quantum yield for normal conditions of corals and bleached corals, except for the studies of Adzis et al. (2009) and Adzis et al. (2013). These were for *P. damicornis* and *A. formosa* and only at Tioman Island, Malaysia. Thus, this experiments data can be a baseline data for maximum quantum yield (dark adapted) data for normal condition and bleached corals of *S. pistillata, M. digitata* and *S. hystrix* regardless in the field or laboratory. The baseline data can help researchers and authorities to monitor any stress events occurring in Malaysian reefs and document it for further studies in the future.

Chlorophyll fluorescence is the main indicator of the photosynthesis process in algae, and it is a very sensitive parameter. Thus, the best way to measure its capacity is the use of PAM fluorometry, to assess the stress levels of the symbiont algae under short thermal shock experiments. These thermal shock treatments are to determine the corals physiological capacity which can relate to their intial thermal stress tolerance during bleaching events. This study exposed three common Malaysian corals; *S. pistillata, M. digitata* and *S. hystrix,* to three treatments: 1. high temperature and high light stress, 2. high temperature and ambient light stress, and 3. ambient temperature and high light stress. The chlorophyll measurement of quantum yield of the corals, after a dark-adaptation method by using Water-PAM fluorometer is measured before experiment, after 3-h stress treatments and after 24-h recovery stage.

Hypothesis

1. The high-ambient to high temperature compared to ambient temperature treatment will decrease the maximum quantum yield of chlorophyll fluorescence (F*v*/F*m*) for all the coral species.
2. Changes in the photosynthetic capacity of the corals species may be seen in the recorded maximum quantum yield of corals before and after stress.
3. Coral species may changein their physical appearance due to stress, becoming pale in colouration and die.

Specific objectives:

1. To record maximum quantum yield of chlorophyll fluorescence (F*v*/F*m*) of coral species; *S. pistillata, M. digitata and S. hystrix* in stress treatments of high-ambient to high temperature compared to ambient temperature treatment.
2. To differentiate changes in quantum yield fluorescence among the three corals species before stress, after 3-h stress treatments and after 24-h recovery stage.
3. To record the changes in physical appearance of each coral species before and after the stress treatments.

5.2 Materials and Methods

5.2.1 Control aquarium set up

Experiments were performed in the laboratory using nubbins (4-5 cm) of three species of scleractinian corals, *Stylophora pistillata*, *Montipora digitata* and *Seriatopora hystrix.* These corals were chosen because of their high and moderate susceptibility to bleaching, fast growth and abundance in Malaysian waters (Loh et al., 2001; Mazlan et al., 2005). The corals were maintained in a 100 L seawater tank in the laboratory with sea water flow-through (3100 L/h) at 27°C (as shown in Figure 4.2), under a light intensity of 200 µmol quanta $m^{-2}s^{-1}$, on a 10h light /14h dark cycle (Warner et al., 1999). The tank was heated by a heater control unit. Metal halide lamps were used in these experiments. Data loggers were put in the tank to track temperature. For maintenance, the tank was cleaned once a week to prevent algal growth.

The coral colonies were sourced from a sustainable culture facility (as explained in Chapter 3) and were acclimated to the controlled conditions for 1 month. Small fragments, 4-5 cm in size, were mounted on cement plugs which are stable for the fragile organisms to be moved from and to experimental tanks.

5.2.2 Experimental protocol for photochemical efficiency measurement

Before and after treatments, the mounted coral nubbins were put in a separate black container box, covered with black canvas, for 20 minutes dark-adapted photochemical efficiency measurement (F*v*/F*m*) (Hoegh-Guldberg and Jones, 1999) using a pulse-amplitude-modulation (PAM) chlorophyll fluorometer (WATER-PAM, Walz, Effeltrich, Germany). The black container box was filled with seawater sufficiently to allow the fluorometer probe to orientate between the corals. All the coral nubbins were mounted on their cement plugs in a vertical position. Measurements were conducted at the surface point of each branch of the nubbins (as shown in Figure 4.3). Healthy coral nubbins were transferred from controlled conditions directly into experimental tanks by putting them carefully in a seawater-filled container. Temperatures in the experimental tanks were stabilized for 3 h prior to experiments using a heater control unit. For the experiment, 3 aquaria (50 l each) were used for each treatment as replicates. All experimental aquaria were aerated by spreader bars to keep the water evenly heated (checked using a thermometer). To examine possible synergistic effect of temperature and light on the photosynthetic performance of phototrophic endosymbionts in the corals, the corals were chosen randomly (by picking up any 15 corals) from the control tank and exposed to 4 treatments of normal and elevated temperature and irradiance for 3 hours. In total, 5 coral pieces were used in each of three replicates of the 4 treatments (n=5). The treatments were: (a) ambient (27°C, 200 µmol quanta $m^{-2}s^{-1}$), (b) high light (27ºC, 520 µmol quanta $m^{-2}s^{-1}$), (c) high temperature (30°C,

200 µmol quanta $m^{-2}s^{-1}$) and (d) high temperature + high light (30°C, 520 µmol quanta $m^{-2}s^{-1}$). The high temperature level reflected those consistently reported *in situ* during bleaching events (Jones 1997). High irradiance levels are based on levels from Hoegh-Guldberg and Jones (1998) and Bhagooli and Hidaka (2004).

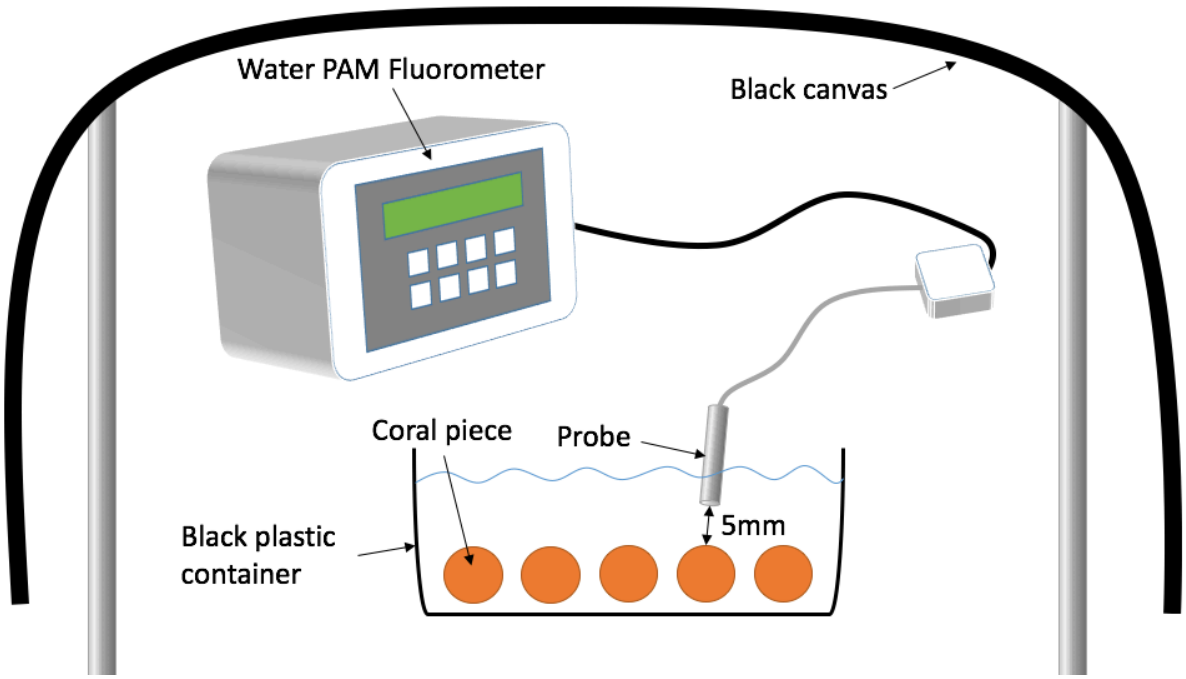


Figure 5.2: Dark-adaptation (20 minutes) chlorophyll fluorescence measurement were undertaken for the coral pieces before stress, after stress and after 24-hour recovery stage to measure its maximum quantum yield (F_v/F_m).

After the stress-experiments for 3 hours, corals were brought to a shallow black water-filled container under a black canvas. Both the black container and canvas allowed the corals to dark-adapt (Bhagooli and Hidaka, 2002) and successive measurements of the F_v/F_m parameter were made. Corals were placed 2-3 cm below the surface of the water. The aquarium received a continuous supply of seawater (at 27 °C, heated by air in controlled aquarium room) and was

provided with gentle aeration. The shallow depth tank was used because the Water-PAM only has 10cm of waterproof probe. The probe was placed on the corals in the water, so as to avoid air-water signal dispersion on a surface parallel to the water surface, as recommended by Hennige et al. (2011). After 20 minutes dark-adapted, the ratio of variable to maximal fluorescence (F_v/F_m) was measured on each coral. All measurements were taken on the vertical sides of tissues, 3 cm from touching the corals (Jones and Hoegh-Guldberg, 2001) to minimize within-branch variability in photosynthetic pigments (Anthony and Hoegh-Guldberg, 2007). Measurements on corals were taken on shaded area to reduce light adaptation of coral as mentioned by Okamoto et al. (2005). The locations of measurement before and after stress were taken at the same place (the locations were refered to a drawn diagram before the experiments) to avoid variable values across the nubbins (Smith, 2018). The initial fluorescence (F_o) was measured by applying a weak pulsed red light (LED 650 nm, 0.6 kHz, 3µs). A saturating pulse of bright actinic light (8000 µmol photons m^{-2} s^{-1}, width 800 ms) was then applied to give the maximal fluorescence value (F_m). Variable fluorescence (Fv) was calculated as $F_m - F_o$ and maximal quantum yield as F_v/F_m (Ferrier-Pagès et al., 2007).

The F_v/F_m of each nubbin was measured in the control tank just before the stress, at the end of the 3-h stress, and after 24 h of recovery. For the following (24 h) stress, paling and mortality of the corals were also recorded. Before the start of the stress experiments, the physiological appearance (colouration) were also examined to compare with the after 24h stress by using CoralWatch chart colour.

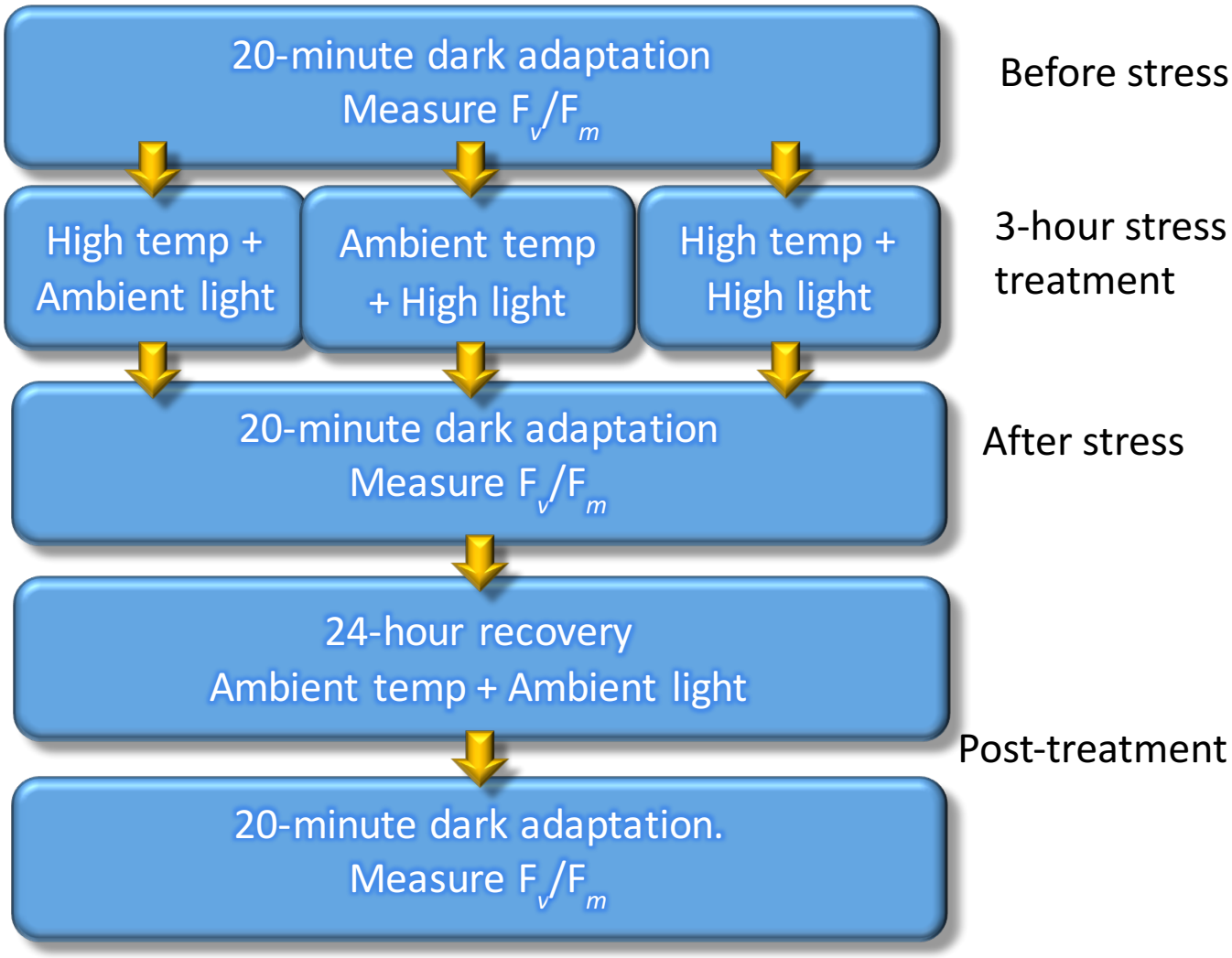


Figure 5.3: Flowchart of maximum quantum of yield measurement for stress treatments of ambient and high temperature combined with ambient and high light for all the coral species.

The short-term shock experiments are so called because the corals were put straight into 30°C from a normal temperature. The objective is to mimic the bleaching response on the short time within 3 hours time. It is a rapid assessment of bleaching vulnerability though a physiological shock experiment. The irregulaties of bleaching patterns timing, the degree and duration of bleaching (Mydlarz et al., 2010).

5.2.3 Data Analysis

Three-way analysis of variance (3-way ANOVA) was used for maximum quantum yield measurements (F_v/F_m) comparisons before stress, after stress and after recovery for each species and among treatments. If the results of the experiments were not significant, data was used in one-way ANOVA to increase the power to detect treatment effects. The post hoc Tukey is also used for multiple comparison of means at $P<0.05$.

5.3 Results

5.3.1 F_v/F_m on healthy corals

Maximum quantum yield (F_v/F_m) of healthy and unstressed corals were measured prior to experiments to establish a normal measurement. A few measurements of surface and side parts of the coral branches were made except for *S. hystrix,* where only surface parts of the coral branches were recorded. This is due to its thin, fragile branches and small size, since side measurements would give a physical impact to the coral nubbins. For both species of the *S. pistillata* and *M. digitata* corals species, surface and side measurements showed a significant result (from ANOVA analysis) ($P<0.05$). Sample 'coral 10' for *S. pistillata* showed a large error bar for its surface measurement, while, sample 'coral 2' of *M. digitata* demonstrated a large error bar for the surface measurement.

As can be seen in the Figure 5.4, F_v/F_m for control samples of *S. pistillata*, readings were of 0.3 to 0.6 for both surface and side parts. For control samples of *M. digitata* corals (Figure 5.5), its Fv/Fm reading was between 0.4-0.55 for both parts of the coral nubbins. For *S. hystrix* (Figure 5.6), its control reading F_v/F_m was much lower, which is between 0.25 to 0.45.

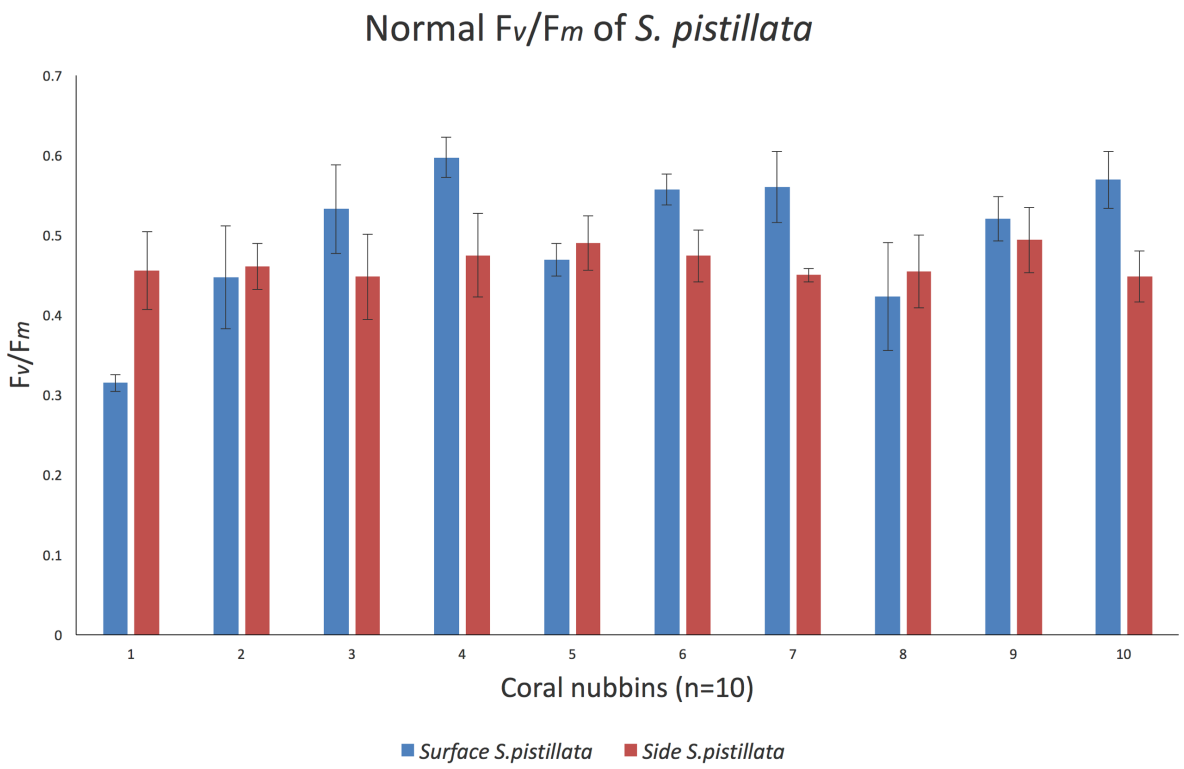


Figure 5.4 The normal yield measurement of maximum quantum yield (*Fv/Fm*) on surface and side of nubbins of coral species *S. pistillata*.

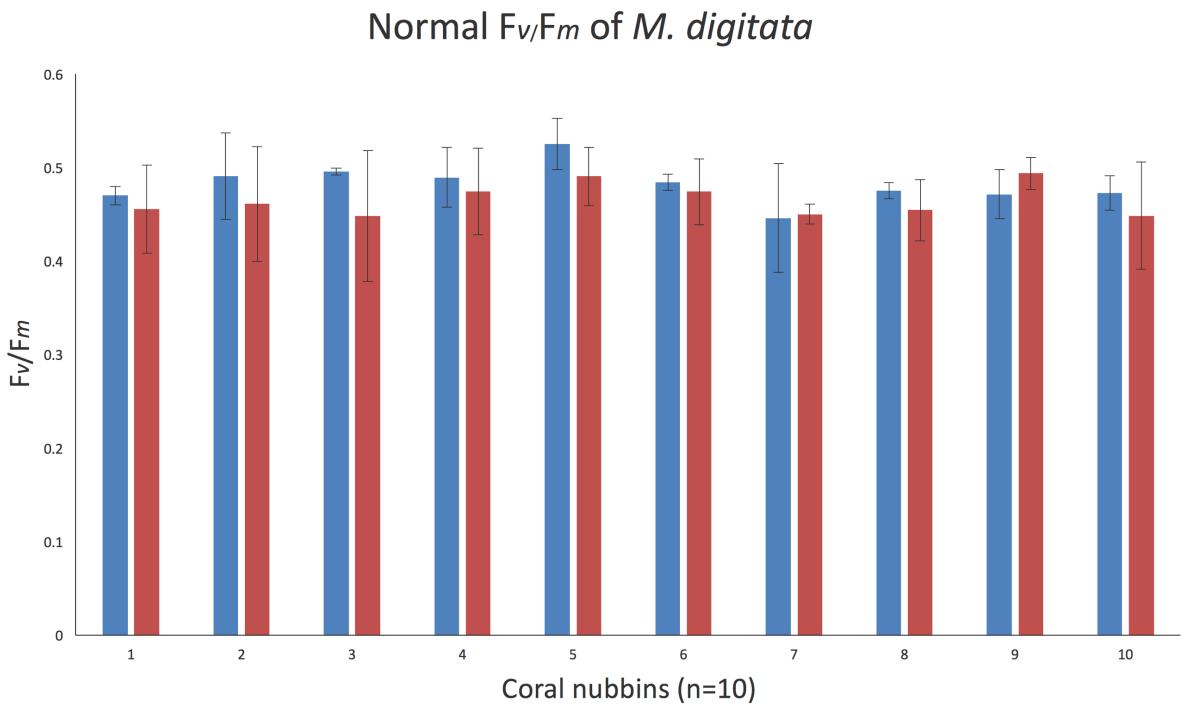


Figure 5.5 The normal yield measurement of maximum quantum yield (F_v/F_m) on surface and side of nubbins of coral species *M. digitata*.

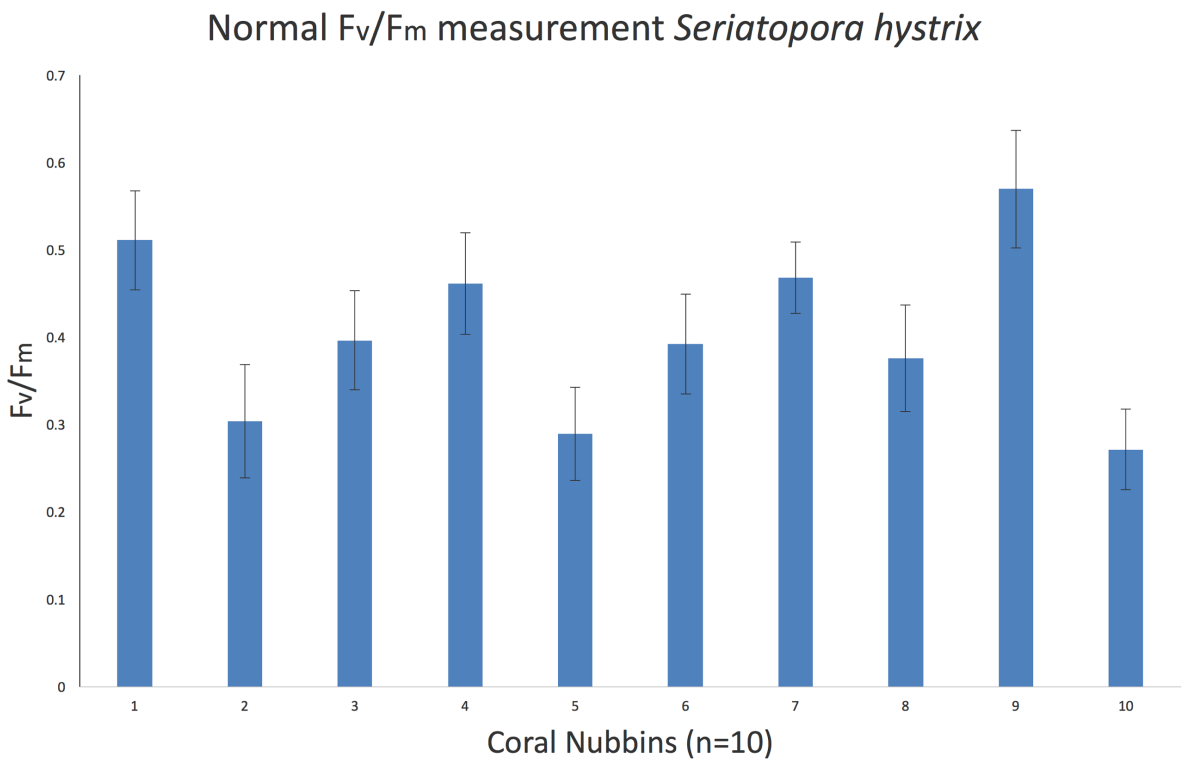


Figure 5.6 The normal yield measurement of maximum quantum yield (F_v/F_m) on surface of nubbins of coral species *S. hystrix*.

5.3.2 Effects of different temperature and light levels on the corals species

Results are presented in Table 5.1 and 5.2. The analysis is a complex, three-way interaction among species, temperature and light after 3-h stress and after 24-h recovery. When data of *S. pistillata* after stress experiments was analysed, it showed that temperature and then the combination of temperature and light did not have any significant effects (Table 5.1) on the measurements, but irradiance did have a significant effect (Table 5.1). The same result for coral species *M. digitata* were achieved, only light stress had reduced the Fv/Fm. (Table 5.2). Meanwhile for *S. hystrix*, the combinations of temperature and light showed a reduction maximum quantum yield (Table 5.3).

The mean for all species in all treatment experiments demonstrated a value between 40-60% of healthiness after 3-h stress (Table 5.4). After stress experiment, *S. hystrix* had lower values of Fv/Fm compared to both *S. pistillata* and *M. digitate*.

The 24-h post-treatment condition results (Table 4.2), showed that temperature and light or the combinations of these did not give any reductions in the maximum quantum yield.

Table 5.1: Results of two-way ANOVA on the effects of 3-h stress treatment on the maximum quantum yield measurement (F*v*/F*m*) of *S. pistillata*, *M. digitata* and *S, hystrix* ($p<0.05$).

Coral species	Source	df	Mean Square	F	Sig.
S. pistillata	temp	1	365.398	.901	.347
	light	1	3142.126	7.744	.008
	temp * light	1	220.055	.542	.465
M. digitata	temp	1	2.596	.006	.938
	light	1	1507.179	3.547	.073
	temp * light	1	223.913	.527	.476
S. hystrix	temp	1	2910.410	5.725	.023
	light	1	2718.441	5.347	.028
	temp * light	1	456.621	.898	.351

Table 5.2: Results of two-way ANOVA on the effects after recovery of 3-h stress treatment on the maximum quantum yield measurement (F*v*/F*m*) of *S. pistillata*, *M. digitata* and *S, hystrix* ($p<0.05$).

Coral species	Source	df	Mean Square	F	Sig.
S. pistillata	temp	1	644.614	1.466	.232
	light	1	848.851	1.930	.171
	temp * light	1	706.437	1.606	.211
M. digitata	temp	1	18.516	.031	.862
	light	1	1747.196	2.937	.101
	temp * light	1	18.516	.031	.862
S. hystrix	temp	1	1150.734	2.674	.112
	light	1	682.019	1.585	.217
	temp * light	1	572.803	1.331	.257

Table 5.3: The descriptive statistics for after stress (F_v/F_m before stress with F_v/F_m after stress) and recovery period (different values of F_v/F_m after stress with F_v/F_m after 24-h recovery) for three coral species were tested using Tukey Multiple Comparisons test. Statistically significant results ($P<0.05$) are highlighted in bold font.

Dependent Variable	Species	Species	Sig.
AFTER	*M. digitata*	*S. hystrix*	**.032**
		S. pistillata	.998
	S. hystrix	*M. digitata*	**.032**
		S. pistillata	**.006**
	S. pistillata	*M. digitata*	.998
		S. hystrix	**.006**
RECOVERY	*M. digitata*	*S. hystrix*	.794
		S. pistillata	.949
	S. hystrix	*M. digitata*	.794
		S. pistillata	.901
	S. pistillata	*M. digitata*	.949
		S. hystrix	.901

Temperature and light stress among the species

For all the stress experiments, all showed a slightly lower recovery bar graph compared to the control experiment, except for *S. hystrix* during high temperature and high light stress, *M. digitata* and *S. hystrix* during high temperature and ambient light stress. These two-species showed a small degree of recovery during the dark-adapted chlorophyll fluorescent measurement after the stress experiment.

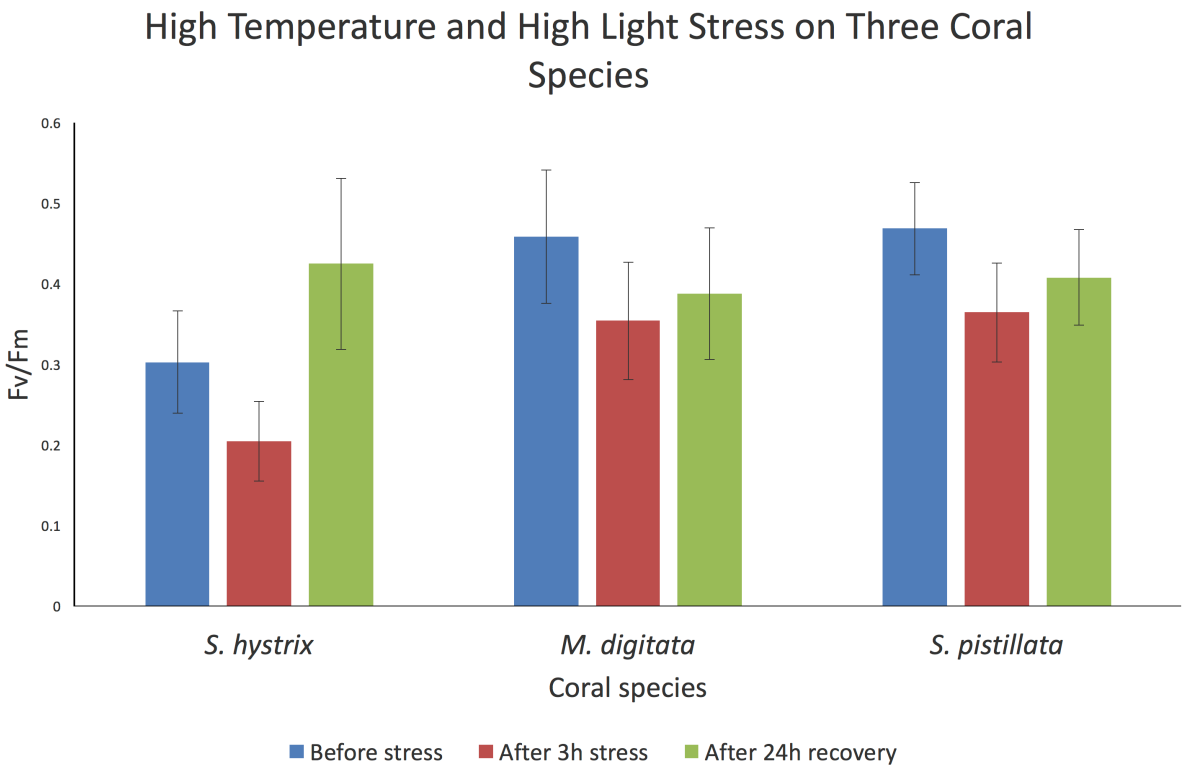


Figure 5.7: Maximum quantum yield, F*v*/F*m* before stress, after stress and after recovery for each species during treatment at high temperature and high light. The chlorophyll fluorescence of corals was measured after 20-min of dark-adaptation for each stage of before, after stress and after recovery of 24h.

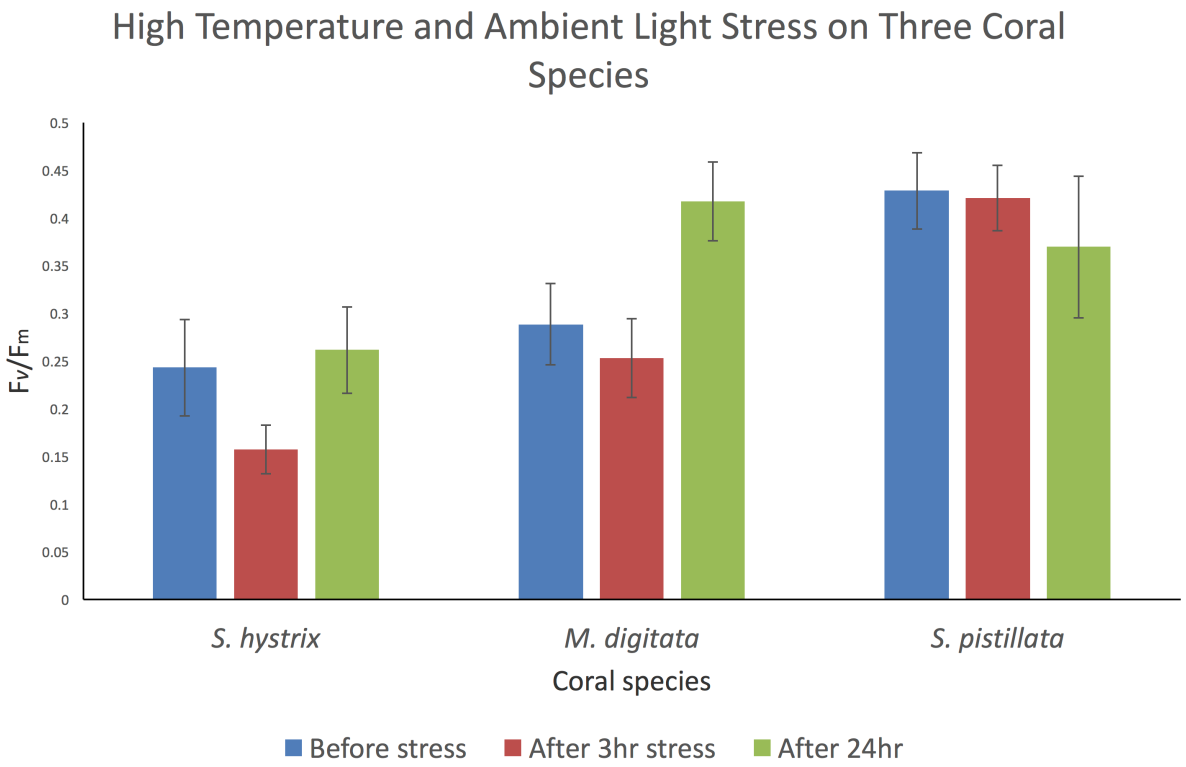


Figure 5.8: Maximum quantum yield, F*v*/F*m* before stress, after stress and after recovery for each species during treatment at high temperature and ambient light. The chlorophyll fluorescence of corals was measured after 20-min of dark-adaptation for each stage of before, after stress and after recovery of 24h.

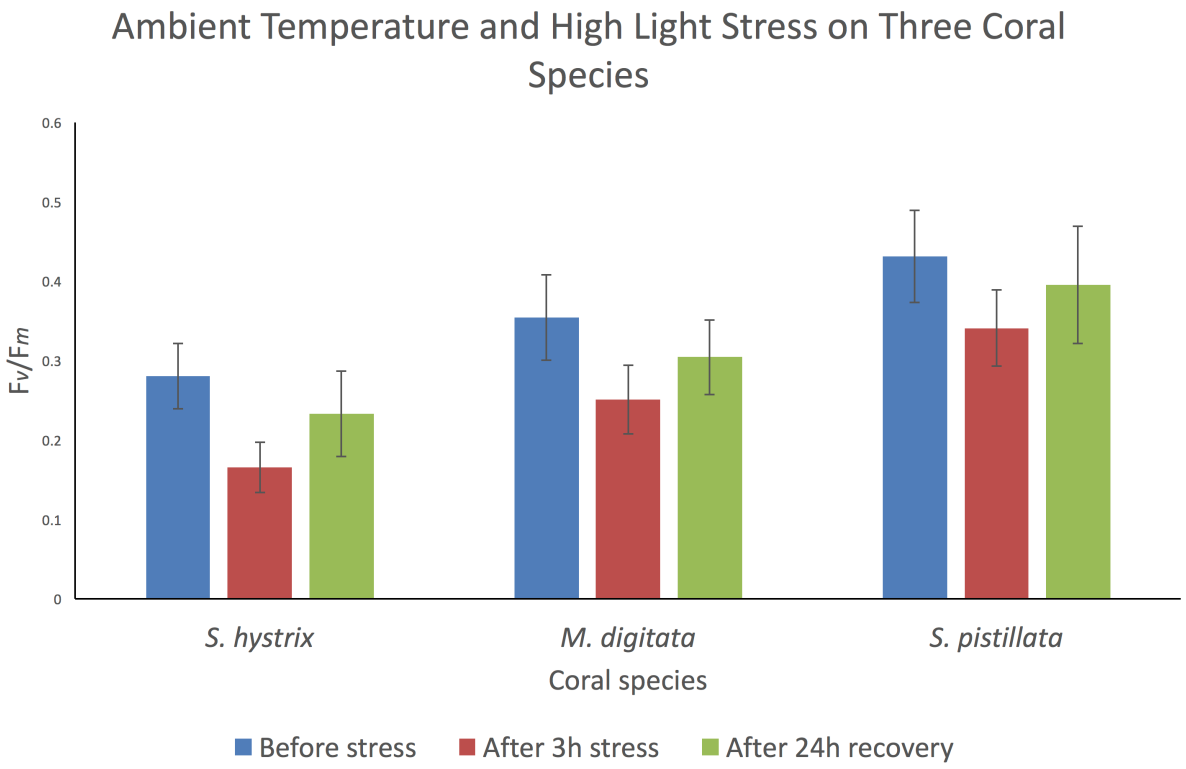


Figure 5.9: Maximum quantum yield, F*v*/F*m* before stress, after stress and after recovery for each species during treatment at ambient temperature and high light. The chlorophyll fluorescence of corals was measured after 20-min of dark-adaptation for each stage of before, after stress and after recovery of 24h.

5.3.3 Bleaching conditions and mortality of the coral species

After 24-h in a state of recovery, following a 3-h stress for all treatments, *S. pistillata* and *M. digitata* did not show any pale colours and mortality, by naked-eye visualization. During the stress treatments, the corals retracted their polyps, but did not show any colour changes, after stress and after 24-h recovery. But, 9 out of 15 coral nubbins of *S. hystrix* were completely bleached after the treatment stress of high temperature and high light. They started to bleach

from the tips of the nubbins during the recovery stage. By 48 hours, 9 of the brown coral nubbins turned completely white (as shown in Figure 5.13).

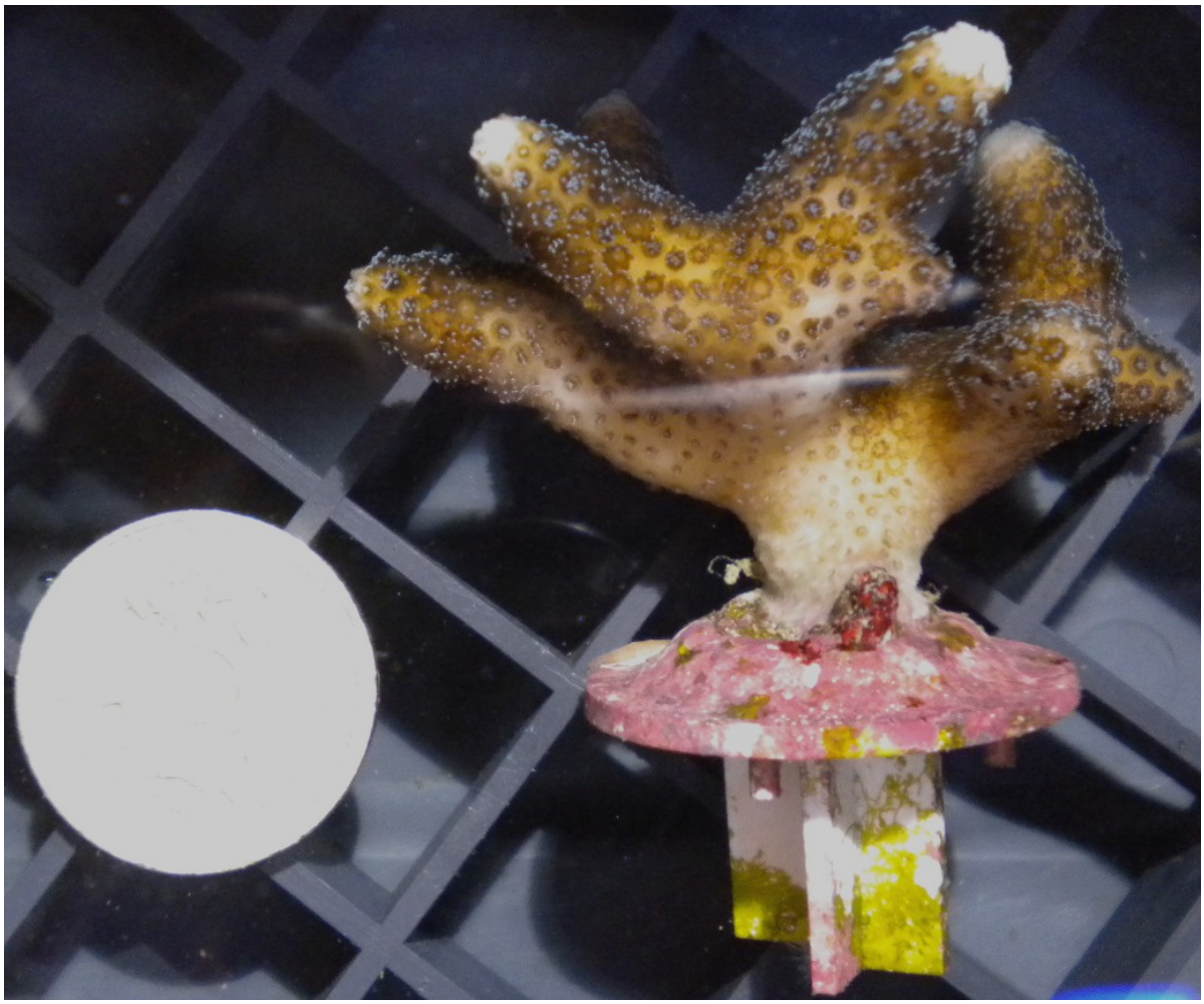


Figure 5.10: Healthy *S. pistillata*

Figure 5.11: Healthy *M. digitata*

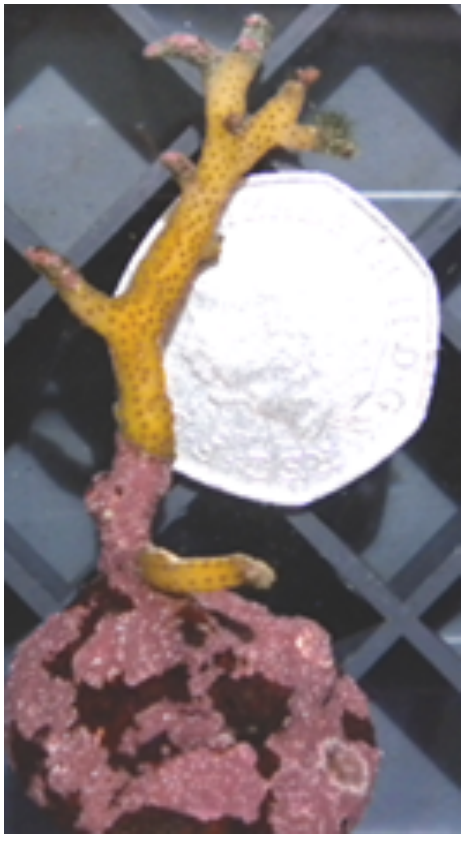


Figure 5.12: Healthy *S. hystrix*

Figure 5.13: White corals were the bleached colonies of *S. hystrix* and the brown corals were the unbleached colonies of *S. hystrix*.

5.4 Discussion

This chapter aimed to investigate the potential thermal tolerance of three Scleractinian corals and changes in the physiology of corals. The rationale for assessing thermal tolerance is that thermal bleaching (caused by sea surface temperature) begins with the process of photoinhibition (Baird et al., 2008). The magnitude changes in irradiance and temperature (heat stress), either on their own or the combinations of the factors, may increase the susceptibility of the photosynthetic pigment to photoinhibit within the stressed corals by blocking the ability to process excitations captured by the light reactions of photosynthesis (Fine et al., 2004).

5.4.1 Different bleaching susceptibility

These are three species of corals with different stress susceptibility that have been used in this study. They have a wide distribution range and are common species of Indo-Pacific shallow waters. Warm seawater temperature and high irradiance have been documented to cause a bleaching in many coral communities and later followed by death of all or part of the many species colonies (Hoegh and Jones, 1999). But, different hermatypic coral species responded in different ways to bleaching (Stimson et al., 2002). *S. pistillata* is categorized as a complex and robust species, and has been a classic-studied specimen for symbiosis, physiology, cell biology, the calcification process and photosynthesis (Karako-Lampert et al., 2014). *S. pistillata* and *S. hystrix* was once demonstrated to have survived bleaching in tanks (Stimson et al., 2002). During the bleaching event in summer 1998 of northern Okinawa waters, *S. pistillata* and *S. hystrix* were recorded as suffering high mortality but a high percentage of colonies of *M. digitata* survived the bleaching (Stimson et al., 2002). But *M. digitata,* is known as a bleaching-sensitive coral: Marshall and Baird (2000) studied highly damaged of *M. digitata* in Great Barrier Reef,

Yamazato (1981) recorded bleached *Montipora* in Sesoko Station, Okinawa and Sugihara et al. (1999) reported similar result for *M. digitata* in Ryukyu Islands. Changes to Fv/Fm have been used to examine bleaching response previously. Fine et al. (2004) reported that the maximum quantum yield (using Diving-PAM Walz, Germany) of the non-bleached colony of coral *Oculine patagonica* were significantly higher (between 0.6-07 value of F_v/F_m) (between 0.3-0.4 value of F_v/F_m) than the bleached corals. Even after long exposure (one year) to high irradiances, the colonies did not recover from bleaching, when the endolithic algae showed low photosynthetic level (there is absent zooxanthellae) (Fine et al., 2004).

5.4.2 Effects of temperature and light towards corals

Elevated temperatures and irradiance have been implicated as causes for the loss of symbiotic algae in corals and other invertebrates with photoautotrophic symbionts (Lesser, 1996). The results of the present study clearly show that each of the coral species had a different response towards three stress treatments and one control. All the coral species demonstrated a decrease of F_v/F_m in all treatments of temperature and light stresses, meaning that the corals underwent photoprotective processes to dissipate extra-excitation energy out from PSII, photodamage or both (Bhagooli and Hidaka, 2003). In this study, the high light factor did not significantly affect both *S. pistillata* and *M. digitata.* Previous studies by Warner et al (1999), Jones and Hoegh-Guldberg (2001) and Bhagooli and Hidaka (2003), reported that high irradiance levels may cause the process of bleaching in corals.

The third species studied here, *S. hystrix,* also made a reaction significantly to light, but combined with temperature. It clearly shows that the three corals species exhibited photoinhibition when there is incoming light and the capacity of a photosystem to process the protons is relatively decreased (Long et al., 1994, Osmond 1994; Hoegh-Guldberg and Jones,

1999). Photoinhibition is synonymous with light-related decreases in the dark-adapted measurement of maximum quantum yield in plants where there are changes in photosynthetic electron transport activity and in chlorophyll fluorescence characteristics (Jones and Hoegh-Guldberg, 2001). The level of irradiances that was used in this study is 200 µmol quanta $m^{-2}s^{-1}$ (as the ambient treatment) and 520 µmol quanta $m^{-2}s^{-1}$ as high light treatment. Bhagooli and Hidaka (2004) also reported similar results for its samples to respond significantly towards PAR fluxes of 520 µmol quanta $m^{-2}s^{-1}$. At high light levels, photosynthetic pigment usually takes lighter than can be used for the photosynthesis process, and it can cause photodamage, and loss in photosynthetic productivity (Smith et al., 2005).

This study highlighted the photosynthetic response of three coral species towards combinations of high and ambient of seawater temperature, and high and ambient irradiance. Only *S. hystrix* showed a significant effect towards high temperature and high light stress. Other species, *S. pistillata* and *M. digitata* did not show any response to the stress treatments. High temperature and high light levels used in this study are 30°C and 520 µmol quanta $m^{-2}s^{-1}$, respectively. Photosynthetic pigment exposed to temperatures above 30°C shows a high level of lipid in the thylakoid membrane which generates ROS and PSII damage (Jones 1997; Hoegh-Guldberg and Jones, 1999) (Flores and Linnan, 2006). This study only focused on the F_v/F_m value after stress and after recovery, apart from visualization on the physiology of the coral nubbins. There was a decrease of F_v/F_m value after stress for all treatments. According to Iglesias-Prieto et al., (1992); Lesser 1996, Jones et al., (1998); Warner et al., (1999), one of the earliest photosynthesis processes in response to thermal stress is the decrease of its maximum quantum yield. A decrease in F_v/F_m value has been applied as a good indicator to monitor photoinhibition of photosynthesis (Nakamura and Yamasaki, 2008). Suggested by Jones et al (1998), high-light treatment only cannot exacerbate bleaching onto corals, but there must be interactions among temperature, light

and time in the bleaching response, in accordance with photoinhibition. The photoinhibition process during this stress is included in the heat block dark reaction (Calvin cycle) and excess light caused damage to PSII due to the formation of reactive oxygen species (Bhagooli and Hidaka, 2004).

5.4.3 Bleaching conditions and mortality of the coral species

After the extreme stress, 60% of *S. hystrix* nubbins were visually bleached after the 24-h recovery stage. The other two species did not show any sign of bleaching and mortality during the 24-h recovery stage. Baird et al (2008) mentioned that *Stylophora* spp., *Montipora digitata (Pocillopora* spp.) and *S. hystrix* are among the species that are highly susceptible to bleaching. Bhagooli and Hidaka (2003) described similar results with the order of susceptibility in the field as *S. pistillata>M. digitata>Platygrya ryukyuensis>Galaxea fascicularis*, *Psammocora contigua*. But this study did not support this finding because *S. pistillata* and *M. digitata* were not affected by any of the stress experiments. Only the response of *S. hystrix* supported Baird et al., (2008) which was found to be sensitive toward thermal stress. The coral showed a significant reduction of photosynthetic efficiency (F_v/F_m) towards the temperature of 30°C and irradiance level of 520 µmol quanta $m^{-2}s^{-1}$ (Bhagooli and Hidaka, 2003). After 24-h recovery, most of *S. hystrix* coral nubbins had lost all photosynthetic pigment colour. There are many discussions on the paling of bleached corals, either the bleaching process is caused by the decreased amounts of photosynthetic pigments of the zooxanthellae or the decreased numbers of the zooxanthellae (Hoegh-Guldberg and Smith, 1989). In this study, by using the methodology of measuring the chlorophyll *a* fluorescence with PAM fluorometer, it is suggested that bleaching is caused by the disruption process in the photosynthetic pigments of the corals' zooxanthellae. Temperature and light, either acting independently or together, are known to be the most important stressors for coral bleaching and mortality (Glynn, 1996 and Brown 1997) (D'Croz and Mate, 2004). *S.*

hystrix in this study exhibited their bleaching response caused by the combination of temperature and irradiance stress. Higuchi et al (2009) indicated that elevated seawater temperature significantly decreased not only the coral photosynthesis, but disturbed the coral's calcification and increased its respiration rates. The bleaching process will give impacts on coral's energy reserves for maintenance and growth, where its lipid stores were depleted during the process, and this lead to increased risk of mortality (Anthony and Hoegh-Guldberg, 2007). Reduced energy in bleached corals can also affect its reproductive activity, where the coral lost up to 90% of zooxanthellae population and the majority of their food energy source. The reproduction of the corals was severely affected by mass bleaching in 1998 in Heron Island (Ward et al., 2000). Zooxanthellae type in the corals gives roles for the thermal tolerance of the coral species (Berkelmans and Oppen, 2006). There are corals with clade D symbionts can tolerate more with thermal stress compared to corals with clade C symbionts (Sugget et al., 2017). *Pocillopora* which recruited more thermal tolerant zooxanthellae were confirmed to be more abundant and more resilient in the mass bleaching events (Murphy et al., 2018). In this study, zooxanthellae types were not studied but there are possibilities of different coral species has different zooxanthellae clades which has different bleaching susceptibility.

Further studies related to corals' calcification, growth, respiration rates and other molecular studies should be done to relate the effect of photoinhibition in the coral species.

5.5 Conclusion

The effect of different combinations of temperatures and irradiances on photosynthetic quantum yield of three Scleractinian corals species was demonstrated. This short thermal shock experiments show a decrease of maximum quantum yield of chlorophyll *a* fluorescence measurement for all species, which is the response of the highly susceptible corals in terms of photoinhibition. High light was the major cause of photoinhibitions in *S. pistillata, M. digitata*

and *S. hystrix*. These results highlighted that different corals have different stress responses, based on the maximum quantum yield after stress treatments and after recovery stage where the combination of high stress of temperature and light only can cause the bleaching effect for *S. hystrix*, whereas *S. pistillata* and *M. digitata* did not have any significant negative effect from those stress treatments. It is important to study the mechanisms of coral bleaching by using short thermal shock experiments to study the initial response of coral species (the symbionts particularly) and how the corals towards incoming coral bleaching events. The response of corals towards stress is might be dependent on the zooxanthellae types, with the zooxanthellae being the weakest link in the symbiotic relationship (Berkelmans and Oppen, 2006). To understand how coral reefs coping with increased thermal challenges is the knowledge of the role of the zooxanthellae in the thermal tolerance of the holobiont (Berkelmans and Oppen, 2006). Importantly is to understand the symbiont behaviour towards increased thermal tolerance in the context of climate change, whether the corals can tolerate to the anticipated increases in sea surface temperatures (SSTs) (Berkelmans and Oppen, 2006).

References

Ainsworth T.D., Hoegh-Guldberg O., Heron S.F., Skirving W.J. and Leggat W. 2008. Early cellular changes are indicators of pre-bleaching thermal stress. *Journal of experimental marine biology and ecology* **364:** 63-71.

Anthony R.N.K., Connoly S.R. and Hoegh-Guldberg O. 2007. Bleaching, energetic and coral mortality risk: Effects of temperature, light and sediment regime. *Limnology Oceanography* **52 (2):** 716-726.

Baird A.H. and Marshall P.A. 2002. Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. *Mar Ecol Prog Ser* **237:** 133–141.

Berkelmans, R. and Van Oppen, M.J., 2006. The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope'for coral reefs in an era of climate change. *Proceedings of the Royal Society of London B: Biological Sciences* 273 **(1599):** pp.2305-2312.

Bhagooli R. and Hidaka M. 2002. Physiological Response of the Coral *Galaxea fascicularis* and Its Algal Symbiont to Elevated Temperatures. *Galaxea. Jcrs* **4:** 33-42.

Bhagooli R. and Hidaka M. 2003. Comparison of Stress Susceptibility of *in hospite* and Isolated Zooxanthellae among Five Coral Species. 2003. *Journal of Experimental Marine Biology and Ecology* **291:** 181-197.

Bhagooli R. and Hidaka M. 2004. Photoinhibition, Bleaching Susceptibility and Mortality in Two Scleractinian Corals, *Platygyra ryukyuensis* and *Stylophora pistillata*, in Response to Thermal and Light Stresses. *Comparative Biochemistry and Physiology Part A* **137:** 547-555.

Borell E.M. 2008. Coral photophysiology in response to thermal stress, nutritional status and seawater electrolysis. PhD Thesis. Bremen.

Borell E.M., Yuliantri A.R., Bischof K. and Richter C. 2008. The Effect of Heterotrophy on Photosynthesis and Tissue Composition of Two Scleractinian Corals Under Elevated Temperature. *Journal of Experimental Marine Biology and Ecology* **364:** 116-123.

Bruno J.F., Selig E.R., Casey K.S., Page C.A., Willies B.L., Harvell C.D., Sweatman H. and Melendy A.M. 2007. Thermal stress and coral cover as drivers of coral disease outbreaks. *PLoS Biology* **5 (6):** 1220 -1227.

Coles S.L. and Jokiel P.L. 1978. Synergistic Effects of Temperature, Salinity and Light on the Hermatypic Coral *Montipora verrucosa. Marine Biology* **49:** 187-195.

D'Croz L. and Matè J.L. 2004. Experimental response to elevated water temperature in genotypes of the reef coral *Pocillopora damicornis* from upwelling. *Coral Reefs* **23:** 473-483.

Ferrier-Pagès C., Richard C., Forcioli D., Allemand D., Pichon M. and Shick J.M. 2007. Effects of Temperature and UV Radiation Increases on the Photosynthetic Efficiency in Four Scleractinian Coral Species. *Biology Bulletin* **213:** 76-87.

Fine M., Steindler L. and Loya S. 2004. Endolithic Algae Photoacclimate to Increased Irradiance During Coral Bleaching. *Marine and Freshwater Research* **55:** 115-121.

Fitt W.K., Gates R.D., Hoegh-Guldberg O., Bythell J.C., Jatkar A., Grottoli A.G., Gomez M., Fisher P., Lajuenesse T.C., Pantos O., Iglesias-Prieto R., Franklin D.J., Rodrigues L.J., Torregiani J.M., van Woesik R. and Lesser M.P. 2009. Response of Two Species of Indo-Pacific Corals, *Porites Cylindrical* and *Stylophora pistillata,* to Short-Term Thermal Stress. The Host Does Matter in Determining the Tolerance of Corals to Bleaching. *Journals of Experimental Marine Biology and Ecology* **373:** 102-110.

Flores-Ramirez and Linan-Cabello M. 2006. Relationship among thermal stress, bleaching and oxidative damage in the hermatypic coral, *Pocillopora capitata*. *Comparative Biochemitry and Physiology, Part C* **146:** 194-202.

Glynn P.W. 1993. Coral Reef Bleaching: Ecological Perspectives. *Coral Reefs* **12:** 1-17.

Gorbunov M.Y., Kolber Z.S., Lesser M.P., Falkowski P.G. 2001. Photosynthesis and Photoprotection in Symbiotic Corals. *Limnology and Oceanography* **46:** 75-85.

Guest J.R., Baird A.H., Maynard J.A., Muttaqin E., Edwards A.J., Campbell S.J., Yewdall K., Affendi Y.A. and Chou L.M. 2012. Contrasting Patterns of Coral Bleaching Susceptibility in 2010 Suggest an Adaptive Response to Thermal Stress. *PLOS One* **7(3):** e33353.

Hader D.P., Kumar H.D., Smith R.C. and Worrest R.C. 2007. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical and Photobiological Sciences* **6:** 267-285.

Hennige S.J., McGinley M.P., Grottoli A.G. and Warner M.E. 2011. Photoinhibition of *Symbiodinium* spp. within the reef corals *Montastraea faveolata* and *Porites astreoides*: implications for coral bleaching. *Marine Biology* **158:** 2515-2526.

Higuchi T., Fujimura H., Arakaki T. and Oomori T. 2009. The synergistic effects of hydrogen peroxide and elevated seawater temperature on the metabolic activity of the coral *Galaxea fascicularis*. *Marine Biology* **156:** 598-596.

Hill R. and Ralph P.J. 2006. Photosystem II Heterogeneity of *in hospite* Zooxanthellae in Scleractinian Corals Exposed to Bleaching Conditions. *Photochemistry and Photobiology* **82:** 1577-1585.

Hoegh-Guldberg O. and Jones R.J. 1999. Photoinhibition and Photoprotection in Symbiotic Dinoflagellates from Reef-Building Corals. Marine Ecology Progress Series vol 183:73-86.

Jones R.J. 1997. Changes in Zooxanthellae Densities and Chlorophyll Concentrations in Corals During and After a Bleaching Event. *Marine Ecology Progress Series* **158:** 51-59.

Jones R.J., Hoegh-Guldberg O., Larkum A.W.D. and Schreiber U. 1998. Temperature-Induced Bleaching of Corals Begins with Impairment of the CO_2 Fixation Mechanism in Zooxanthellae. *Plant, Cell and Environment* **21:**1219-1230.

Jones R.J. and Hoegh-Guldberg O. 2001. Diurnal Changes in the Photochemical Efficiency of the Symbiotic Dinoflagellates (Dinophyceae) of Corals: Photoprotection, Photoinactivation and the relationship to bleaching. *Plant, Cell and Environment* **24:** 89-99.

Karako-Lampert S., Zoccola D., Salmon-Divon M., Katzenellenbogen M., Tambutte S., Bertucci, Hoegh-Guldberg O., Deleury E., Alleman D and Levy O. 2014. Transcriptome Analysis of the Scleractinian Coral *Stylophora pistillata. PLoS ONE* **9(2):** E88615.

Loh W.K., Loi T., Carter D. and Hoegh-Guldberg O. 2001. Genetic Variability of the Symbiotic Dinoflagellates from the Wide-Ranging Coral Species *Seriatopora hystrix* and *Acropora longicyathus* in the Indo-West Pacific. *Marine Ecology Progress Series* **222:** 97-107.

Loya Y., Sakai K., Yamazato K., Nakano Y., Sambali H. and van Woesik R. 2001. Coral Bleaching: The Winners and the Losers. *Ecology Letter* **4:** 122-131.

Marshall P.A. and Baird A.H. 2000. Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. *Coral Reefs* **19:**155-163.

Mazlan A.G., Zaidi C.C., Wan-Lotfi W.M. and Othman B.H.R. 2004. On the Current Status of Coastal Marine Biodiversity in Malaysia. Indian Journal of Marine Sciences vol 34 **(1):** 76-87.

Murphy, J.W., Spies, N.P. and Richmond, R.H., 2018. *Conservation of Symbiodinium spp. clade in the coral Pocillopora damicornis during the 2014 mass-bleaching event* (No. e26514v1). PeerJ Preprints.

Mydlarz, L.D., McGinty, E.S. and Harvell, C.D., 2010. What are the physiological and immunological responses of coral to climate warming and disease? *Journal of Experimental Biology* **213 (6):** pp.934-945.

Nakamura T. and Yamasaki H. 2008. Flicker Light Effects on Photosynthesis of a Symbiotic Algae in the Reef Building Coral *Acropora digitifera* (Cnidaria: Anthozoa: Scleractinia). *Pacific Science* **62 (3):** 341-350.

Smith D.J., Suggett D.J. and Baker N.R. 2005. Is Photoinhibition of Zooxanthellae Photosynthesis the Primary Cause of Thermal Bleaching in Corals? *Global Change Biology* **11:** 1-11.

Stimson J., Sakai K. and Sembali H. 2002. Interspecific Comparison of the Symbiotic Relationship in Corals with High and Low Rates of Bleaching-Induced Mortality. *Coral Reefs. Springer-Verlag* **21:** 409-421.

Suggett, D.J., Warner, M.E. and Leggat, W., 2017. Symbiotic dinoflagellate functional diversity mediates coral survival under ecological crisis. *Trends in ecology & evolution* 32 **(10):** pp.735-745.

Strychar and Sammarco. 2009. Exaptation in coral to high seawater temperatures: Low concentration of apoptotic and necrotic cells in host coral tissue under bleaching conditions. *Journal of Experimental Marine Biology and Ecology* **369:** 31-42.

Warner M.E., Fitt W.K. and Schmidt G.W. 1999. Damage to Photosystem II in Symbiotic Dinoflagellates: A Determinant of Coral Bleaching. *Proc. Natl. Acad. Sci. USA* **96:** 8007-8012.

Ward S., Harrison P. and Hoegh-Guldberg O. 2000. Coral Bleaching Reduces Reproduction of Scleractinian Corals and Increases Susceptibility to Future Stress. Proceedings 9th International Coral Reef Symposium, Bali, Indonesia. 23-27 October 2000.

Takahashi S., Nakamura T., Sakamizu M., van Woesik R. and Yamasaki H. 2004. Repair machinery of symbiotic photosynthesis at the primary target of heat stress for reef-building corals. *Plant cell physiology* **45 (2):** 251-255.

Ulstrup K.E., Hill R. and Ralph P.J. 2005. Photosynthetic impact of hypoxia on *in hospite* zooxanthellae in the scleractinian coral *Pocillopora damicornis. Marine ecology progress series* **286:** 125-132.

Winters G., Loya Y., Röttgers R. and Beer S. 2003. Photoinhibition in shallow-water colonies of the coral *Stylophora pistillata. Limnology Oceanography* **48 (4):** 1388-1393.

Chapter 6

Response of Scleractinian corals to nitrate enrichment in high and ambient seawater temperatures

6.1 Introduction

Coral-zooxanthellae symbioses have the ability to survive in a low-nutrient environment, unlike any other organisms (Stanley, 2003). This is because they have an effective way of recycling the dissolved nitrogen in the symbiotic association (Muscatine et al. 1981; Falkowski et al. 1993). The low concentration of inorganic nutrients in the reef ecosystem means low primary production by symbiotic algae (Sakka et al., 2002). The productivity of a coastal ecosystem is very dependent on nutrient distribution and behaviour and the nutrient concentration is in large part due to the entry of domestic and industrial waste, urban drainage and agricultural effluents from the terrestrial ecosystem (Praveena et al., 2013). Elevated nutrient concentrations surrounding coral reef ecosystems can affect the function and composition of the zooxanthellae photosynthesis process (Rodríguez-Román and Iglesias-Prieto, 2005) and it can cause the photosynthetic algae to alter its protein composition, increase in chlorophyll *a* specific absorption coefficients and reduce maximum quantum yields of PSII (Rodríguez-Román and Iglesias-Prieto, 2005).

The growth of symbiotic algae on coral reefs can function well in a nitrogen-limited condition, allowing the coral to maintain its symbiont population density (Stanley, 2003). Water quality is very important to a sensitive organism, such as coral, so any negative disturbances on the water quality in its ecosystem will lead to the degradation of the coral communities (Tan et al., 2008).

A study of coastline pollution in West Malaysia, identified oil palm plantations as the cause of nitrate and phosphorus enrichment in the sediment and nutrient loads of rivers and coastal waters (Jakobsen et al., 2007). Combined nitrification, global warming and loss of top members of the food chain occurred over the last 65 million years (Knowlton, 2001). Nutrification is one of the most important contributors to reef decline (Risk, 1999; Hallock, 2005), creating a 'phase shift' from a coral dominanted environment to one of mixed coral-algal domination (Hallock, 2005). Human activities increase the flux of nutrient into coastal waters causing a complex process of organic production and accumulation and change in the nutritional status of the community (Nixon, 1995; Szmant, 2002). Coastal regions often receive large anthropogenic inputs of nitrogen that cause eutrophication (Herbert, 1999) by human activities such as agriculture and aquaculture (Suratman and Latif, 2014). The degradation of coral reefs worldwide is in part caused by urbanization in coastal areas, which has led to intensive agricultural activities, and rains have carried the sediment and nutrients from fertilizer into the water (Koop et al., 2001).

This nutrient input and sediment loading caused serious impacts to coral reefs (Cortes and Risk, 1985), suppressing calcification and stimulating algal growth (Tomascik and Sander, 1985; Tomascik, 1990; Marubini and Davies, 1996). Loya (2004) showed that 50% of coral mortality was from benthic algal blooms, and reduced coral calcification was caused by nutrient increase from fish farms in the Northern of Gulf of Aqaba, Red Sea. In Hong Kong, Morton (1994) and Hodgson and Yau (1997) studied that excess pollutants, nutrients and sediment dredging had decreased coral recruitment. Nutrient enrichment reduced coral cover and increased the macroalgal cover in Kanehoe Bae, Hawaii (Stimson et al., 2001) and on the Great Barrier Reef (van Woesik et al., 1999). Brazilian nearshore reefs received substantial nutrients from surface runoff, submarine groundwater discharge and untreated sewage, which had enhanced algal growth, which leads to competition for space with corals (Costa et al., 2008).

The study by Marubini and Davies (1996) found that increasing nitrate concentration in the water (1, 5 and 20 μM) caused an increase in the population density of zooxanthellae, chlorophyll *a* , and protein (c_2) per cell. Marubini and Atkinson (1999) showed that an increase in nitrate did not affect the corals' growth at all, in term of calcification rates, after being exposed to 0.5 – 5.0 μM to corals in five weeks. In a study of nitrate limitation in symbiotic dinoflagellates, photochemical efficiency of PSII (F_v/F_m) values were negatively correlated with external nitrogen concentrations in the culture of *Symbiodium kawagutii* and *S. pilosum* (Rodríguez-Román and Iglesias-Prieto, 2005).

Malaysia is situated in Southeast Asia, comprising of 30% of the world's coral reef, but in 1992 (ASEAN-Australia Living Coastal Resources project) 60% of it was destroyed (Wilkinson et al., 1993). One of the causes of reef destruction in Southeast Asia is organic and inorganic pollution (Lundin and Linden,1993), with concentrations of particulate organic nitrogen (PON), ranging from <10 to 131 μM found in Malaysian creek waters, while dissolved organic nitrogen (DON) were ranging between 20-50 μM (Nixon et al. 1984).

6.1.1 Justification of this study

Nutrient input in Malaysian waters caused by coastal development, sewage discharge and sedimentation, need to be managed to let the corals reefs survive in Malaysian waters (Reef Check Malaysia, 2014). Somchit et al., (2009) believed that the organic chemicals pollution that occurs in Malaysia originated from urbanization (sewage waters, industrial), agricultural activities (nutrients, pesticides and sediment) and transporting (boating and shipping). That was based on a critical review of levels of persistence organic chemicals in Malaysian water from

1980 to 2002. Even though Nutrient Indicator Algae (NIA) was considered low (3.92%) in the surveyed islands in Malaysia in 2016, it should be noted that at some sites, the proliferation of algae is an issue and needs to be taken care of (ReefCheck, 2016). NIA is the indication of the health of herbivorous fish and the invertebrates, and the level of nutrient input to reefs (ReefCheck, 2016). Bong and Lee (2008) reported that dissolved inorganic nutrients (ammonium, nitrite, nitrate, phosphorus and silicate) were high in nearshore areas of West Coast, Peninsular Malaysia, due to anthropogenic activities affecting the marine water quality threatening the recreational industry, tourism, fisheries and biodiversity. Involvement of stakeholders and government for is required for better management, ensuring more frequent surveys, better enforcement of existing laws of regulating activities and also community involvement to help the coral reef management strategies to address local threats. In Malaysia, there are no studies relating to the nitrate levels with coral bleaching and fluorescent measurement (using PAM fluorometer). There are only studies on PAM fluorometer measurements with before-after tsunami and coral bleaching events (Abdul-Adzis, 2009). This study is specifically designed to examine the synergistic effects of ambient/high temperature levels combined with ambient/high nitrate levels on three common Indo-Pacific coral species, *S. pisitillata, M. digitata* and *S. hystrix* by data collection on maximum quantum yield (F_v/F_m) using PAM fluorometer.

Hypothesis

1. The combined stress of elevated temperature and nitrate levels, will reduce the mean maximum quantum yield of chlorophyll fluorescence (F_v/F_m) for all the coral species.
2. A decrease in photosynthetic capacity will cause a change in their colouration during the post-treatment stage; after high temperature and high nitrate treatments.

Specific objectives:

1. To record maximum quantum yield of chlorophyll fluorescence (F*v*/F*m*) of coral species; *S. pistillata, M. digitata and S. hystrix* in stress treatments of high temperature and ambient nitrate levels, ambient temperature and high nitrate levels, and high temperature and high nitrate levels.
2. To differentiate changes in quantum yield fluorescence among the three corals species before stress, after 5-h stress treatments and after 24-h post-treatment stage; temperature-nitrate stress treatments.
3. To record the changes in physical appearance (paling and polyp reaction) of each coral species after the temperature-nitrate stress treatments and after 24-hour recovery.

6.2 Experimental protocol for photochemical efficiency measurement

Experiments were performed in the laboratory using nubbins (4-5 cm) of three species of scleractinian corals, *Stylophora pistillata*, *Montipora digitata* and *Seriatopora hystrix*. They were chosen because of their high and moderate susceptibility to bleaching, fast growth and abundance in Malaysian waters (Mazlan et al., 2005; Loh et al., 2001). The corals were maintained in a 100 L seawater tank in the laboratory at 27°C, under a light intensity of 200 µmol quanta $m^{-2}s^{-1}$, on a 10h light /14h dark cycle (Warner et al., 1999). The tank was heated by a heater control unit using two metal halide lamps. The coral colonies were sourced from a sustainable lab (details in Chapter 3) and were acclimatized to the maintenance conditions in the laboratory aquaria. Small fragments, 4-5 cm in size, were mounted on cement plugs providing stability for the fragile organisms to be moved from and to experimental tanks.

Before and after treatments, the mounted coral nubbins were put in a separate black container box, covered with black canvas, for 20 minutes dark-adapted photochemical efficiency measurement (F*v*/F*m*) (Hoegh-Guldberg and Jones, 1999) using a pulse-amplitude-modulation (PAM) chlorophyll fluorometer (WATER-PAM, Walz, Effeltrich, Germany). The black container box was filled with seawater high enough to allow the fluorometer probe to orientate between the corals. All the coral nubbins were mounted on their cement plugs in a vertical position. Measurements were conducted on surface point of each branch of the nubbins.

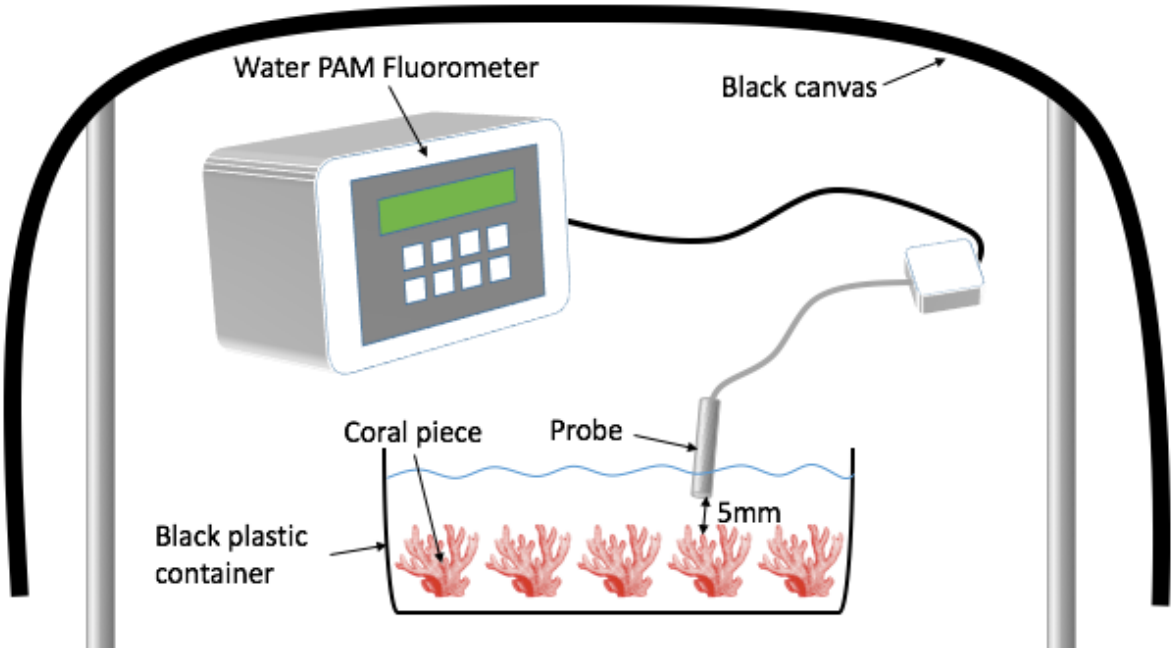


Figure 6.1: Measurement of F_v/F_m onto corals by Water-PAM fluorometer under a black canvas for a dark adaptation measurement, recording maximum quantum yield before stress, after stress and after 24-hr post-treatment stage.

Healthy coral nubbins were transferred from controlled conditions into experimental tanks. They were collected and straightaway put into a small cup underwater in the control tanks and moved to the experimental tank. Temperatures in the experimental tanks were stabilized for 3 h prior to experiments using a heater control unit. For the experiment, 3 aquaria (50 l each) were used for each treatment as replicates. All experimental aquaria were aerated by spreader bars to keep the water evenly heated.

In this experiment, nitrate was added to the nutrient enriched treatments as $NaNO_3$. Nitrate was used in this study because it is the main form of nitrogen available in tropical waters besides ammonium and phosphate (Marubini and Davies, 1996). Ambient laboratory concentration was maintained between 1.0 - 2.0 µM as per other studies (eg. Nordermar et al., (2003) recorded 1.4 – 2.1 µM is ambient nitrate concentration in a laboratory condition). The corals were exposed

to 3 treatments of normal and elevated temperature and nitrates. In total, 4 treatments of normal and elevated temperature and irradiance for 3 hours. In total, 5 coral pieces were used in each of three replicates of the 4 treatments (n=5). The treatments were:

(a) ambient control (27ºC, 2 µM NO_3^-)

(b) high nitrate (27ºC, 15 µM NO_3^-)

(c) high temperature (30ºC, 2 µM NO_3^-)

(d) high temperature + high nitrate (30ºC, 15 µM NO_3)

After the stress-experiments, corals were brought to a shallow black water-filled container under a black canvas. Both black container and canvas allowed the corals to dark-adapt and made successive measurements of the F_v/F_m parameter of the samples (Bhagooli and Hidaka, 2002). Corals were placed 2-3 cm below the surface of the water in a shallow tank because the Water-PAM only has 10cm of waterproof probe. The probe was placed within 5mm of the corals in the water, so as to avoid air-water signal dispersion on a surface parallel to the water surface, as recommended by Hennige et al. (2011). After 20 minutes of dark-adaption, the ratio of variable to maximal fluorescence (F_v/F_m) was measured on each coral. All measurements were taken on the vertical sides of tissues, 3 cm along from the branch tip (Jones and Hoegh-Guldberg, 2001). This also minimized within-branch variability in photosynthetic pigments (Anthony and Hoegh-Guldberg, 2007). The initial fluorescence (F_o) was measured by applying a weak pulsed red light (LED 650 nm, 0.6 kHz, 3µs). A saturating pulse of bright actinic light (8000 µmol photons m^{-2} s^{-1}, width 800 ms) was then applied to give the maximal fluorescence value (F_m). Variable fluorescence (Fv) was calculated as $F_m - F_o$ and maximal quantum yield as F*v*/F*m* (Ferrier-Pagès et al., 2007).

The F_v/F_m of each nubbin was measured in the control tank just before the stress, at the end of the 5-h stress, and after 24 h of recovery. For the following (24 h) stress, paling and mortality of the corals were also recorded. Coral colours were compared using a Coral Health Card by CoralWatch.

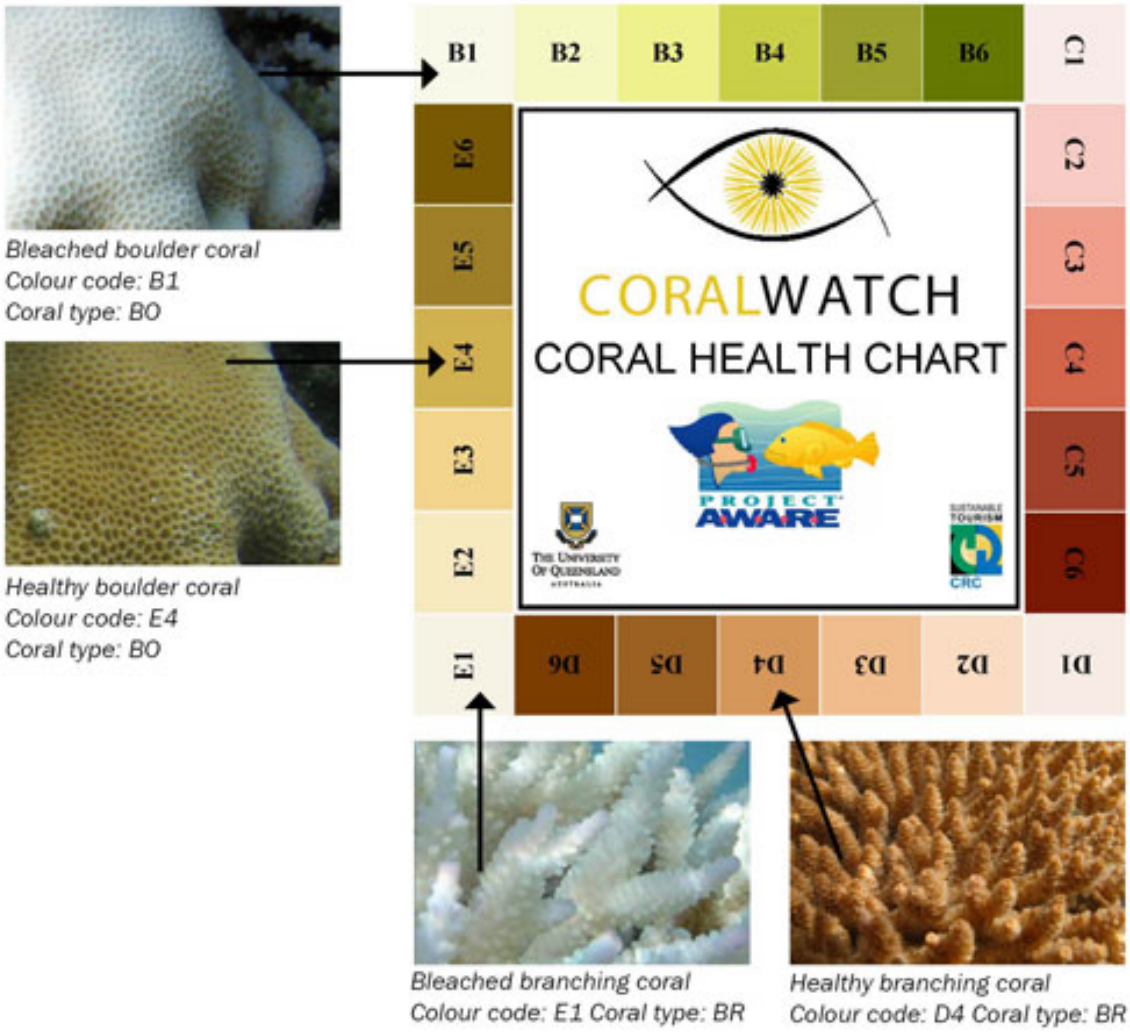


Figure 6.2: Coral colour before and after stress were compared with CoralWatch Coral Health Chart by CoralWatch.org.

6.1.2 Data Analysis

Three-way analysis of variance (3-way ANOVA) was used for comparisons of F*v*/F*m* before stress, after stress and after recovery for each species and among treatments. If the results of the experiments were not significant, the data was used in one-way ANOVA to increase the power to detect treatment effects. The post-hoc Tukey is also used for multiple comparison of means at $P<0.05$.

6.3 Results

6.3.1 Effects of different temperature and nitrate levels on the corals species

Figure 6.2 shows the data of F_v/F_m for ambient temperature and high nitrate of stress (27ºC, 15 µM NO_3^-) for the three corals, *S. pistillata*, *M. digitata* and *S. hystrix* before stress, after 5-h stress and after 24-h of recovery period. After stress and after 24-hour recovery data for *S. pistillata* and *S. hystrix* coral nubbins did not differ during the recovery stage. From the graph, coral species displayed a lower level of maximum quantum yield after a dark adaptation soon after 5 hours in stress experiments.

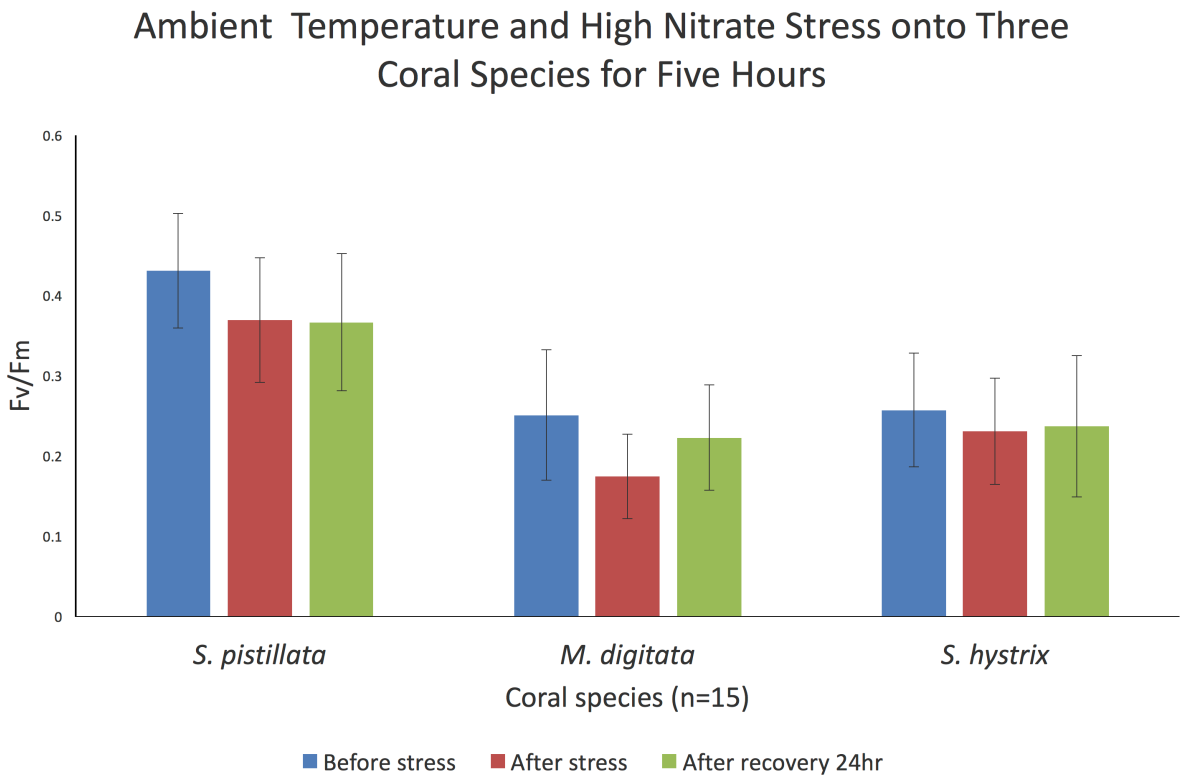


Figure 6.3: Maximum quantum yield measurements for three coral species before stress, after 5-h stress and after 24-h recovery stage during ambient temperature and high nitrate stress treatments.

For the second experiment (Figure 5.3), of high temperature (30ºC) and ambient nitrate level (2 µM NO_3^-), all corals species were affected by the stress by showing some lower level of Fv/Fm measurement soon after 5-hr stress. However, after a 24-hr recovery period, all the corals species showed an increased level of maximum quantum yield compared to the after-stress data. Post-Tukey data analysis gives the results for mean difference among coral species before the stress, after 5-hr stress and after 24-hr recovery. For before stress condition, only the interaction between *S. hystrix* and *S. pistillata* were significantly different ($p<0.05$), but not for *S. pistillata* vs *M. digitata, M. digitata* vs *S. hystrix.* For after stress and after recovery, all of the coral species were not significant with each other.

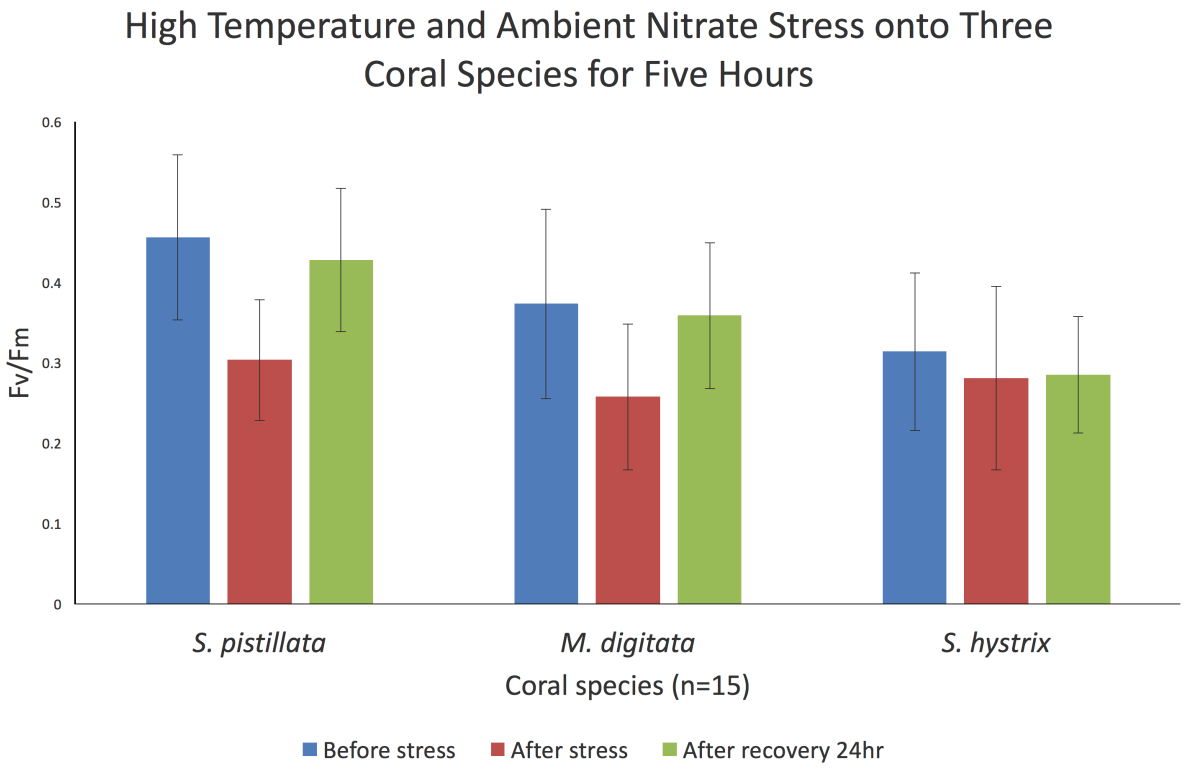


Figure 6.4: Graph of maximum quantum yield measurements for three coral species before stress, after 5-h stress and after 24-h recovery stage during high temperature and ambient nitrate stress treatments.

Table 6.1: Multiple comparison ANOVA for F_v/F_m mean values of *S. pistillata*, *M. digitata* and *S. hystrix* during before stress, after 5-hr stress and after 24-hr recovery period for high temperature and ambient nitrate stress treatments.

Dependent Variable	(I) SPC	(J) SPC	Std. Error	Sig.
beforestress	M. digitata	S. hystrix	.045979	.630
		S. pistillata	.043631	.173
	S. hystrix	M. digitata	.045979	.630
		S. pistill	.044943	.022
	S. pistillata	M. digitata	.043631	.173
		S. hystrix	.044943	.022
afterstress	M. digitata	S. hystrix	.038196	.091
		S. pistillata	.036246	.471
	S. hystrix	M. digitat	.038196	.091
		S. pistillata	.037335	.563
	S. pistillata	M. digitata	.036246	.471
		S. hystrix	.037335	.563
Post-treatment	M. digitat	S. hystrix	.04012711	.906
		S. pistillata	.03807800	.229
	S. hystrix	M. digitat	.04012711	.906
		S. pistillata	.03922252	.108
	S. pistillataa.	M. digitata	.03807800	.229
		S. hystrix	.03922252	.108

In the 3rd experiment (Figure 5.4), of high temperature and high nitrate level stress treatments (30ºC, 15 µM NO_3^-), the yield measurement for the three corals species show a similar situation where there is a decrease after stress, and for after-24hr recovery, the yield increased when compared to after 5-h stress. Table 6.2 shows the results of a statistical test on the effects of stress treatment and recovery on the Fv/Fm values of the coral species. For before stress and after recovery period, Fv/Fm values of *S. pistillata, M. digitata* and *S. hystrix* did not give any significant effect for the treatment.

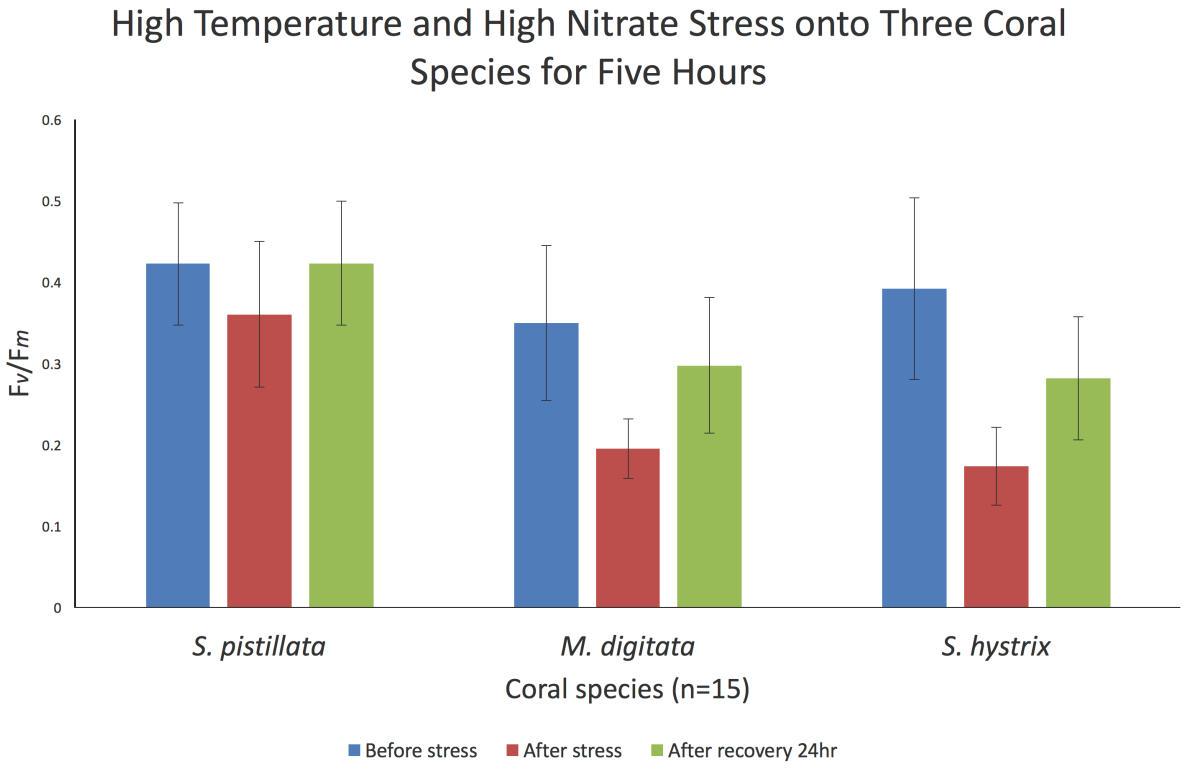


Figure 6.5: Graph of maximum quantum yield measurements for three coral species before stress, after 5-h stress and after 24-h recovery stage during high temperature and high nitrate stress treatments.

Table 6.2: Multiple comparison ANOVA for F_v/F_m mean values of *S. pistillata*, *M. digitata* and *S. hystrix* during before stress, after 5-hr stress and after 24-hr recovery period for high temperature and high nitrate stress treatments.

Dependent Variable	(I) SPC	(J) SPC	Std. Error	Sig.
beforestress	M. digitata	S. hystrix	.066154	.510
		S. pistillata	.067711	1.000
	S. hystrix	M. digitata	.066154	.510
		S. pistillata	.062925	.458
	S. pistillata	M. digitata	.067711	1.000
		S. hystrix	.062925	.458
afterstress	M. digitata	S. hystrix	.065003	.018
		S. pistillata	.066533	.019
	S. hystrix	M. digitata	.065003	.018
		S. pistillata	.061830	.999
	S. pistillata	M. digitata	.066533	.019
		S. hystrix	.061830	.999
post-treatment	M. digitata	S. hystrix	.065223	.797
		S. pistillata	.066758	.276
	S. hystrix	M. digitata	.065223	.797
		S. pistillata	.062040	.584
	S. pistillata	M. digitata	.066758	.276
		S. hystrix	.062040	.584

6.3.2 Maximum quantum yield ratio after 5-h treatments

Table 6.3: Three-way analysis of variance (3-way ANOVA) of maximum quantum yield by temperature and nitrate for three coral species following 5-h treatments.

Source	Dependent Variable	df	Mean Square	F	Sig.
spc	afterstress	2	.446	17.149	.000
	afterrecov	2	.377	12.549	.000
temp	afterstress	1	.064	2.471	.117
	afterrecov	1	.037	1.235	.267
nitrate	afterstress	1	.323	12.419	.000
	afterrecov	1	.227	7.560	.006
spc * temp	afterstress	2	.194	7.451	.001
	afterrecov	2	.049	1.641	.196
spc * nitrate	afterstress	2	.078	3.005	.051
	afterrecov	2	.016	.550	.578
temp * nitrate	afterstress	1	.281	10.816	.001
	afterrecov	1	.160	5.323	.022
spc * temp * nitrate	afterstress	2	.022	.853	.427
	afterrecov	2	.010	.346	.708

spc: species, temp: temperature, afterstress: after 5-h stress treatments, afterrecov: after 24-h recovery period.

Table 6.4: Significant differences of maximum quantum yield measurements among coral species of *S. pistillata, M. digitata* and *S. hystrix* after stress, for all treatments. Means difference were tested using Tukey multiple comparison test.

Dependent Variable		spc	spc	Std. Error	Sig.
afterstr	Tukey HSD	M. digitata	S. hystrix	.02317	.008
			S. pistillata	.02192	.000
		S. hystrix	M. digitata	.02317	.008
			S. pistillata	.02230	.080
		S. pistillata	M. digitata	.02192	.000
			S. hystrix	.02230	.080

Significant test: $P<0.05$

Afterstr: after 5-h stress treatments, spc: coral species.

A three-way ANOVA was run on 15 coral nubbins (n=5) to examine the effect of temperature, nitrates and species on the maximum quantum yield. There was no significant three-way interaction. For two-way interactions of temperature and nitrate treatments during after stress and after recovery, there was a significant interaction ($p = .01$) and ($p = .022$).

6.3.3 Maximum quantum yield ratio after 24-h recovery

Table 6.5: Summarized results of significant (S) and not significant (NS) for each treatment during after stress and post-stress treatment for all three species.

Species	Treatments	Ambient temp High Nitrate	High temp Ambient Nitrate	High temp High Nitrate	Ambient temp Ambient Nitrate
S. pistillata	After stress	S	S	S	S
	Post-treatment	NS	NS	NS	S
M. digitata	After stress	NS	S	S	S
	Post-treatment	NS	NS	NS	S
S. hystrix	After stress	NS	S	S	S
	Post-treatment	NS	NS	NS	S

Maximum quantum yield for all species after stress and after recovery were analysed for each species for all treatments. Ambient temperature and ambient nitrate is the control experiment. After recovery values of F_v/F_m for all species were not significantly different for all stress experiments. In after stress conditions, all coral species showed a significant value of F_v/F_m during high temperature and ambient nitrate; high temperature and high nitrate stress experiments. F_v/F_m values of coral *M. digitata* and *S. hystrix* were not significantly affected by stress treatments of ambient temperature and high nitrate levels. Only *S. pistillata* were significantly stressed after treatment of ambient temperature and high nitrate levels.

6.3.4 Visual bleaching and mortality after stress treatments

In the stress experiment of ambient temperature and high nitrate, coral species responded differently in terms of paling and bleaching by comparing coral colours with CoralWatch Coral Health Chart before and after stress. For high temperature and high nitrate stress experiments, *S. pistillata* showed no paling colours or bleaching after the experiments. But, all coral nubbins of *M. digitata* and *S. hystrix* showed retracted polyps and pale colours immediately after the 5-h experiments where pale colours coded as C4 for *M. digitata* and D3 for *S. hystrix*. But they fully recovered to normal conditions after 24 hours. For high temperature and ambient nitrate stress experiments, 7 of the *S. hystrix* coral nubbins (out of 15) showed paling condition (the most paling colours coded as D3), but recovered after the 24-hour recovery stage. While three coral nubbins of *M. digitata* had retracted polyps after the stress experiment, they returned to normal condition after the recovery stage. *S. pistillata* were normal in colour throughout all the stress treatment experiments. There was no obvious "outward" physiological effect after recovery period.

6.4 Discussion

6.4.1. Coral species response towards high temperature

In all treatments, the quantum yield measurements (F_v/F_m) showed a decrease after the stress experiments, and a subsequent increase after 24-hour recovery adaptation, based on Figure 5.2, 5.3 and 5.4, when compared to the experimental controls (ambient temperature and ambient nitrate) corals. All of the coral species showed a significant result for after stress treatments of high temperatures. In the stress treatment of ambient temperature and high ambient nutrient

levels after the recovery period, all of the three corals species showed non-significant changes in maximum quantum yield measurement. But after stress, only *S. pistillata* coral nubbins responded significantly to the treatments (ambient temperature, high nitrate levels), but not *M. digitata* and *S. hystrix.* The results indicate that high temperature is the dominant stressor to the coral species and the corals are more susceptible to high temperatures (30°C) rather than the high nitrate levels. These findings are similar to those of Humanes et al. (2016), who showed that the temperature stress alone can cause impact to the coral *Acropora tenuis*, not in combination with high nitrate levels. An elevated temperature of 32°C reduced gamete fertilization, larval survivorship and larval settlement of the coral (Humanes et al., 2016). Elevated temperature of 33°C decreased rates of photosynthetic efficiency of PSII in *S. pistillata* in the study which examined the effects of separate and combined increases in temperature and pH towards the uptakes rate of ammonium, nitrate and phosphate by *S. pistillata* corals over 10 days (Godinot et al., 2011). A further study showed that both the density, and F_v/F_m of symbiotic dinoflagellates at 32°C (high temperature) and 20ppt (low salinity) was reduced, compared to an ambient level of temperature and low salinity (26°C and 20ppt) (Xiubao et al., 2009). The high temperature was suggested to be the antagonistic effect between temperature and low salinity (Xiubao et al., 2009).

6.4.2 Coral species respond towards nitrate stress

However, Faxneld et al. (2010) found that high temperature (31°C) combined with nitrate enrichment and decreased salinity could lead corals to rapid mortality, and suggested a synergistic effect. When *Turbinaria mesenterina* was exposed to ambient temperature (25°C) with nitrate enrichment (+5 μM NO_3^-) plus low salinity (20ppm), the corals did not show any effects on metabolism and survival (Faxneld et al., 2010). Synergistic effects of high temperature

(30°C) and high nitrate levels (20 µM NO_3) were also the factors for the reduction of zooxanthellae population and increased cellular chlorophyll in *P. damicornis* and *P. lobata* (Schlöder and D'Croz, 2004). Nordermar et al. (2003) suggested that corals on nutrient-exposed reefs may be more stressed when the combined effects of nitrate enrichment and elevated seawater temperature reduced the gross primary production rate of zooxanthellae. Higuchi et al. (2015) suggested that coral bleaching can be accelerated when *M. digitata* were exposed to high temperatures (32°C) and high nitrate concentrations (10 µM), rather than only a single stress condition. They reported that the nitrate stressor alone was not sufficient enough to induce stress, based on the F_v/F_m values during the combination of nitrate enrichment and ambient temperature or low light intensity condition (Higuchi et al., 2015). This finding is similar to this present study where the corals species had a none significant value of F_v/Fm during nitrate enrichment with ambient temperature stress treatment.

High nutrient levels have indirect effects on corals (Higuchi, 2015). Nitrate (and also ammonium) are the major source for primary production of marine organisms (Codispoti, 1989) and normally, corals can tolerate low level inorganic nutrient waters, with nitrogen concentrations of 0.3 to 1 µM L^{-1} (Grover et al., 2003). Nitrate is the major form of nitrogen that is present in tropical coastal waters (Marubini and Davies, 1996). Guam Island reefs and Pacific atoll reefs can tolerate to 8 µM and 4 µM, respectively (Kinsey and Davies, 1979). Organisms which contain endosymbiotic zooxanthellae are effected by elevated levels of nutrients e.g. coral colony growth (i.e. coral calcification rate and linear extension) and coral larval settlement (Koop et al., 2001). *Porites porites* and *Montastrea annularis* exhibited decreased calcification because of the nitrate enrichment of 5 and 20 µM (Marubini and Davies, 1996). According to Fabricius (2005), increased dissolved inorganic nitrogen (as nitrate and ammonium) was found to impact zooxanthellae density, increasing the content of nitrogen and chlorophyll *a* per

zooxanthella and photosynthetic rates. Nitrate-enriched condition (17 μM of NH_4^+) proved to have a greater algal standing stock and more than doubled algae densities, which means that the zooxanthellae are nutrient limited (Stimson and Kinzie, 1991).

6.4.3 Paling colours on coral species after stress treatments

In this study, nitrate enrichment used was 15 μM, similar to Nordermar et al. (2003) used 15 μM as high nitrate. Fabricius (2005) had found that high nitrate alone can negatively affect the corals. This study showed that, high nitrate and high temperature; ambient nitrate and high temperature; and high nitrate and ambient temperature significantly affect the F_v/F_m measurement towards all coral species. Ammonium enrichment did not contribute significantly to PSI and PSII yield measurement in high temperature waters of *Turbinaria reniformis* corals (Beraud et al. 2013). Wiedenmann et al. (2012) suggested that the photosynthetic efficiency of zooxanthellae can be decreased under a combination of increased temperature and light stress with imbalanced nutrient levels. They proved that increased levels of DIN (dissolved inorganic nitrogen) combined with temperature and light stress can increased a coral's susceptibility to bleaching. That can be seen in this study, after 5-h stress of combined stressors, *M. digitata* and *S. hystrix* experienced paling colours, an early sign of stressed corals. However, they recovered after 24-h in a controlled condition. For bleaching conditions, stressors can cause the breakdown of coral-algal symbiosis and zooxanthellae loss (Wiedenmann et al. 2012). Paling coral shows a decline in density of the zooxanthellae and less pigments within the zooxanthellae (Hoegh-Guldberg, 1989; Kleppel et al., 1989). The pale appearance is because the cnidarian's calcareous skeleton is showing through the translucent tissues (Buchheim, 2005).

The changes in a coral colour after 5-hr stress can be related to the sensitivity of the corals used in the studies. *S. pistillata, M. digitata* and *S. hystrix* had significantly decreased F_v/F_m for combined high nitrate and high temperature. So, if the corals were exposed in a longer period (compared to only 5-hr stress) to either only high nitrate, or only high temperature, or by combination, they might experience bleaching. *Turbinaria mesenterina* was found to be bleached after being exposed to 24-hour stress of increased temperature (31°C), nitrate enrichment (5 µM), low salinity (20) and the combinations of the stressors (Faxneld et al. 2010). Schlöder and D'Croz, (2004) recorded mortality of *P. damicornis* corals with treated with high nitrate and high temperatures and also pale colours for high temperatures stress treatment. The effect of nutrient enrichment on coral varies depending on the coral species (Schlöder and D'Croz, 2004; Yuen et al., 2008). Lower maximum quantum yield was found in nutrient-enriched samples of *S. pistillata* but not in *Acropora* spp. corals (Yuen et al. 2008). While in Schlöder and D'Croz (2004), 90% of *P. lobata* had survived but only 30% of *P. damicornis* remained healthy after a nitrate enrichment stress treatment. According to Yuen et al. (2008), slow growth corals (*S. pistillata*) can survive longer than the faster growing species (*Acropora* spp.). It is similar to the finding of Schlöder and D'Croz (2004), where slow-growing species (*P. lobata*) can cope better with nitrate enrichment conditions than fast-growing species of *P. damicornis.* In this study, *S. pistillata* had shown normal colours throughout the stress experiments but not *M. digitata* and *S. hystrix*. It can be concluded that the survival level of corals species in this experiment is *S. pistillata* > *M. digitata* > *S. hystrix*, based on paling colour changes of corals after the treatments. It can be related to the previous studies, where slow growth corals (*S. pistillata*) are coping better with the environmental changes than the fast growth corals (*M. digitata* and *S. hystrix*).

This study suggests that the impact on coral health might not be affected by nutrient enrichment, but only high temperatures alone can give a significant result of F_v/F_m values for all coral species. Maximum quantum yield of *S. pistillata*, *M. digitata* and *S. hystrix* were negatively affected by the nitrate enrichment, whether in ambient or high temperatures. There is a suggestion that both elevated temperature and nitrogen enrichment can cause a destabilized coral symbiosis (Nordermar et al., 2003), but this present study showed only high temperature can significantly affect the photosynthetic yield. This study shows that the nitrate enrichment itself does not effect the F_v/F_m significant values, but if the nitrate concentrations increased to a higher level, in a high temperature conditions, it might increase the coral's susceptibility to bleaching (Higuchi et al., 2015). Each coral species showed different responses to nutrient enrichment, in terms of their paling colours and survival. Only *S. pistillata* which had no response in terms of colouration before stress, after stress and after 24-hr recovery period for all stress treatments of ambient/high temperatures combined with ambient/high nitrate levels. This study suggests that the effects of high temperature and ambient/high nitrate on corals varies according to coral species.

This study only examined the maximum quantum yield (F_v/F_m), and in a short period of time. Increased nutrient influxes in coral reefs surrounding have ong-term consequences for the corals. Increased of nutrient levels can reduce the heat stress tolerance on corals which expose the corals to coral bleaching risk (D'Angelo and Wiedenmann, 2014). Nitrate enrichment effect onto coral can give any significant changes in symbiont in a short period of time of days to weeks (Béraud et al., 2013 and Koop et al., 2001), not in hours. Nitrate concentration experiments might contribute as an initial reponse of the symbiont's physiological, which lead to the understanding of corals behaviour to environmental change. The changes in the environmental conditions can affect nitrogen cycling in corals, which are the capacity of shifting within coral microbiome

(Rädecker et al., 2015). Besides as an initial response, a short-term nitrate stress onto corals can be resulted in changes of coral colour and physical appearances.

For future investigation, the synergistic impacts of elevated nitrate concentration with other environmental stressor (light, salinity, water motions etc.) should be further study on a larger dynamic of coral-symbiotic association (e.g. zooxanthellae density, chlorophyll *a* content) and in a longer stress-time.

References

Abdul-Adzis K.A., Amri A.Y., Oliver J. and Mohamed C.A.R. 2009. Effective and maximum quantum yield of the lace coral *Pocillopora damicornis* (Anthozoa: Scleractinia: Pocilloporidae) in Pulau Tioman, Malaysia. *Journal of Science and Technology in the Tropics* **5:** 13-17.

Abdullah A.L., Anscelly A.M., Mohamed J. and Yasin Z. 2016. Conservation of Pulau Payar Marine Park and Optical Remote Sensing Models. KEMANUSIAAN 23 (1): 79-107. Penerbit Universiti Sains Malaysia.

Alongi, D.M., Boto, K.G. and Robertson, A.I. 1992. Nitrogen and Phosphorus Cycles, in Tropical Mangrove Ecosystems (eds A.I. Robertson and D.M. Alongi), American Geophysical Union, Washington.

Badgley B.D., Lipshultz F. and Sebens K.P. 2006. Nitrate uptake by the reef coral *Diploria strigosa*: effects of concentration, water flow and irradiance. *Marine Biology.* **149:** 327-338.

Bhagooli R. and Hidaka M. 2004. Photoinhibition, bleaching susceptibility and mortality in two scleractinian corals, *Platygyra ryukyuensis* and *Stylophora pistillata*¸ in response to thermal and light stresses. *Comparative Biochemistry and Physiology* **Part A 137:** 547-555.

Buchheim J. 2005. Coral reef bleaching. *Journal of Odyssey Expeditions, Tropical Marine Biology Voyages*. 10 pp. PMCid: PMC1739595.

Bythell J. C. 1990. Nutrient uptake in the reef-building coral *Acropora palmata* at natural environmental concentrations. *Marine Ecology Progress Series.* **68:** 65-69.

Béraud, E., Gevaert, F., Rottier, C. and Ferrier-Pagès, C., 2013. The response of the scleractinian coral Turbinaria reniformis to thermal stress depends on the nitrogen status of the coral holobiont. *Journal of Experimental Biology*, pp.jeb-085183.

Costa, O. S., Nimmo, M., & Attrill, M. J. (2008). Coastal nutrification in Brazil: a review of the role of nutrient excess on coral reef demise. *Journal of South American Earth Sciences* ***25*****(2):** 257-270.

Cortes J. and Risk M.J. 1985. A coral reef under siltation stress: Cahuita, Costa Rica. *Bulletin of Marine Science* **36:** 339-365.

Wiedenmann, J., D'Angelo, C., Smith, E.G., Hunt, A.N., Legiret, F.E., Postle, A.D. and Achterberg, E.P., 2013. Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nature Climate Change* 3**(2):** p.160.

Fabricius, K. E. 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Marine pollution bulletin*. **50(2):** 125-146.

Falkowski P.G., Dubinsky Z., Muscatine L., McCloskey L. 1993. Population control symbiotic corals. Ammonium ions and organic materials maintain the density of zooxanthellae. *BioScience*. **43:** 606–611.

Faxneld S., Jörgensen T.L. and Tedengren M. 2010. Effects of elevated water temperature, reduced salinity and nutrient enrichment on the metabolism of the coral *Turbinaria mesentria. Estuarine, Coastal and Shelf Science.* **88:** 482-487.

Ferrier-Pagès, Gattuso J.P., Dallot S., Jaubert J. 2000. Effect of nutrient enrichment on growth and photosynthesis of the zooxanthellae coral *Stylophora pistillata*. *Coral Reefs.* **19:** 103-113.

Godinot C., Houlbrèque F., Grover R. and Ferrier-Pagès C. 2011. Coral Uptake of Inorganic Phosphorus and Nitrogen Negatively Affected by Simultaneous Changes in Temperature and pH. *PLoS ONE* **6(9):** e25024.

Hallock P. 2005. Global change and modern coral reefs: New opportunities to understand shallow-water carbonate depositional processes. *Sedimentary Geology* **175:** 19-33.

Hennige S.J., McGinley M.P., Grottoli A.G. and Warner M.E. 2011. Photoinhibition of *Symbiodinium* spp. within the reef corals *Montastraea faveolata* and *Porites astreoides*: implications for coral bleaching. *Marine Biology* **158:** 2515-2526.

Higuchi T., Yuyama I. and Nakamura T. The combined effects of nitrate with high temperature and high light intensity on coral bleaching and antioxidant enzyme activities. *Regional studies in marine science* **2:** 27-31.

Hodgson G. and Yah E.P.M. 1997. Physical and biological controls of coral communities in Hong Kong. In H.A. Lessions, I.G. Macintyre (Eds), *Proceedings of the Eight International Coral Reef Symposium,* Smithsonian Institute Press, Balboa, Panama, pp. 459-461.

Hoegh-Guldberg O. and Smith G.J. 1989. Influence of the Population-Density of Zooxanthellae and Supply of Ammonium on the Biomass and Metabolic Characteristics of the Reef Corals *Seriatopora hystrix* and *Stylophora pistillata*. *Marine Ecology Progress Series* **57 (2):** 173-186.

Hoegh-Guldberg O. and Smith G.J. 1989. The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. *Journal of Experimental Marine Biology and Ecology* **129:** 279-303.

Humanes A, Noonan S.H.C., Willis B.L., Fabricius K.E. and Negri A.P. 2016. Cumulative Effects of Nutrient Enrichment and Elevated Temperature Compromise the Early Life History Stages of the Coral *Acropora tenuis*. PLoS ONE **11(8):** e0161616.

Jakobsen F., Hartstein N., Frachisse J. and Golingi T. 2007. Sabah shoreline management plan (Borneo, Malaysia): *Ecosystems and Pollution. Ocean & Coastal Management* **50 (1-2):** 84-102.

Juan L. and Maté T. 1997. Experimental responses of Panamanian reef corals to high temperature and nutrients. *Proceedings 8th International Coral Reef Symposium* **1:** 515-520.

Kleppel G.S., Dodge R.E., Reese C.J. 1989. Changes in pigmentation associated with the bleaching of stony corals. *Limnology and Oceanography* **34:**1331-1335.

Kinsey and Davies. 1979. Effects of elevated nitrogen and phosphorus on coral reef growth. *Limnology Oceanography* **24 (5):** 935-940.

Koop K., Booth D., Broadbent A., Brodie J., Bucher D., Capone D., Coll J., Dennison W., Erdmann M., Harrison P., Hoegh-Guldberg O., Hutchings P., Jones G.B., Larkum W.D., O'Neil J., Steven A., Tentori E., Ward S., Williamson J. and Yellowlees D. 2001. ENCORE. The Effect of Nutrient Enrichment on Coral Reefs. Synthesis of Results and Conclusions. *Marine Pollution Bulletin* **42 (2):** 91-120.

Loya Y. 2004. The coral reefs of Eilat – past, present and future: three decades of coral community structure studies. In E. Rosenberg and Y. Loya (Eds), Coral Reef Health and Disease, Springer, Berlin (2004) p. 396.

Lundin, C.G.& Lindén, O. 1993. Coastal Ecosystems: attempts to manage a threatened resource. Ambio 22**(7):** 468-473.

Marubini F. and Davies P.S. 1996. Nitrate Increases Zooxanthellae Population Density and Reduces Skeletogenesis in Corals. *Marine Biology.* **127:** 319-328.

Morton B. Hong Kong's coral communities: Status, threats and management plans. *Marine Pollution Bulletin* **29:** 74-83.

Muscatine L, McCloskey LR, Marian RE (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. *Limnol Oceanogr* **26:** 601–61.

Nixon, S.W., Furnas, B.N., Lee, V., Marshall, N., Ong, J.-E., Wong, C.-H., Gong, W.-K., and Sasekumar, A., 1984. The role of mangroves in the carbon and nutrient dynamics of Malaysia

estuaries. In: Soepandmo, E., Rao, A.N., Macintosh, D.J. (Eds). *Proceedings of the Asian Symposium on Mangrove Environments: Research and Management,* pp. 496-513, University of Malaya and UNESCO, Kuala Lumpur.

Nordermar I., Nyström M. and Dizon R. 2003. Effects of elevated seawater temperature and nitrate enrichment on the branching coral *Porites cylindrical* in the absence of particulate food. *Marine Biology* **142:** 669-677.

Pastore M., 2014. The impact of nitrogen eutrophication on Caribbean coral reefs: A review. *Concept* **Vol. XXXVII.**

Praveena S.M., Siraj S.S. and Aris A.Z. 2012. Coral reef studies and threats in Malaysia: a mini review. *Rev Environmen Sci Biotechnol* **11:** 27-39.

Reef Check Malaysia. 2016. Status of coral reefs in Malaysia, 2015. In RCM Annual Survey Report. Reef Check Malaysia, Kuala Lumpur.

Rodríguez-Román, A. and Iglesias-Prieto, R., 2005. Regulation of photochemical activity in cultured symbiotic dinoflagellates under nitrate limitation and deprivation. *Marine Biology* 146 **(6):** 1063-1073.

Rädecker, N., Pogoreutz, C., Voolstra, C.R., Wiedenmann, J. and Wild, C., 2015. Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends in Microbiology* 23 **(8):** pp.490-497.

Schlöder C. and D'Croz L. 2004. Responses of massive and branching coral species to the combined effects of water temperature and nitrate enrichment. *Journal of Experimental Marine Biology and Ecology* **313:** 255-268.

Schick J.M., Iglic K., Wells M.L., Trick C.G., Doyles J. and Dunlap W.C. 2011. Responses to iron limitation in two colonies of *Stylophora pistillata* exposed to high temperature: Implications for coral bleaching. *Limnology Oceanograhy* 56 **(3):** 813-828.

Stanley, G. D. 2003. The evolution of modern corals and their early history. *Earth-Science Reviews* 60 **(3):** 195-225.

Stimson, J., & Kinzie, R. A. 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. *Journal of experimental marine Biology and Ecology* 153 **(1)**: 63-74.

Stimson J., Larned S.T and Conklin E. 2001. Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalga *Dictyosphaeria cavernosa* in Kaneohe Bay, Hawaii. Coral Reefs **19:** 343−357.

Suratman S. and Latif M.T. 2014. Reassessment of nutrient status in Setiu Wetland, Terengganu, Malaysia. *Asian Journal of Chemistry* 27 **(1):** 239-242.

Tan A.S.H., Ismail, I. A., and Yasin, Z. (2009). Diversity of Family Fungiidae in Malaysian Waters. *Publication of the Seto Marine Biological Laboratory - Special Publication Series* **9:** 15-24.

van Woesik R., Tomascik T. and Blake S. 1999. Coral assemblages and physico-chemical characteristics of the Whitsunday Islands: evidence of recent community changes. *Marine and Freshwater Research* **50:** 427−440.

Wiedenmann J., D'Angelo C., Smith E.G., Hunt A.N., Legiret F-E., Postle A.D. and Achterberg E.P. 2012. Nutrient enrichment can increase susceptibility of reef corals to bleaching. *Nature Climate Change.*

Wilkinson, C.R., Chou, L.M., Gomez, E., Ridzwan, A.R., Soekarno, S. and Sudra, S. 1993. Status of coral reefs in Southeast Asia: Threats and responses. Global Aspects of Corals: Health, Hazard and History. University of Miami.

Yuen Y.S., Nakamura T., Yamazaki S.S. and Yamasaki H. (2008) Long-term effects of inorganic nitrogen enrichment on the reef-building corals *Stylophora pistillata* and *Acropora* spp. *Proceedings of the 11th International Coral Reef Symposium, Florida 7th-11th July 2008.*

Zhu, B., Wang, G., Huang, B. and Tseng, C.K., 2004. Effects of temperature, hypoxia, ammonia and nitrate on the bleaching among three coral species. *Chinese Science Bulletin* 49 **(18):** pp.1923-1928.

Chapter 7

Conclusion

7.1 Summary

7.1.1 Introduction

Coral bleaching is a reaction to abnormal environmental conditions and has been observed to be caused by the variation in physical and chemical parameters (Jones *et al*., 1998). The bleaching condition is caused by the loss of pigmentation in reef-building corals (expelled from the host), which occurs by either decreases in the number of zooxanthellae or in the concentration of photosynthetic pigments in the zooxanthellae (Jones et al., 1998). In the environment, bleaching can be caused by stressors such as changes in salinity, high visible and/or ultraviolet irradiation, increased sedimentation and nutrients or pollutants such as heavy metals, and also including the combination of elevated sea surface temperatures with high solar radiation (Weis, 2008). Climate attribution research found that the human factor impact is more than 90% of the cause of recent mass bleaching episodes on the northern Great Barrier Reef in 2016 (Donner et al., 2017).

Malaysia is one country under the Coral Triangle Initiative, containing 76% of the world's coral species (Tun et al., 2004). Malaysia was affected by the global bleaching events of 1998 and 2010 when corals bleached and recovered, but there was also coral degradation happening after the events (in Tioman, Redang and Perhentian Islands; Langkawi Island and East of Malaysia). Because of the tourism industry in Peninsular Malaysia and Eastern Malaysia, the coral communities of marine parks and other tourism areas (e.g. Port Dickson, Negeri Sembilan;

Langkawi Island) were degraded by sedimentation and effluent discharges (UNEP, 2007). Untreated sewage flows directly into the ocean from the hotels, resorts and chalets in Redang, Tioman and Sibu-Tinggi Islands (UNEP, 2007). Nutrient runoff was reported to be a significant threat to reefs in marine parks all over Malaysia (Harborne et al., 2000).

Chlorophyll fluorescence is an indicator of photosynthetic activity in organisms (higher plants, phytoplankton, algae and bacteria). PAM- fluorometry (Water-PAM specifically) as used in this study, is known for its non-intrusive methodology, and rapid technique, and is widely used for determining phytoplankton photosynthetic efficiency and physiological stress (temperature, salinity and irradiance). Coral species used in this study were adapted to conditions as lab-cultured coral, therefore they are reliable for use in experiments. *Stylophora pistillata, Montipora digitata* and *Seriatopora hystrix* were chosen as experimental samples because they are common Indo-Pacific corals and have different susceptibility towards stress (i.e. bleaching).

The effects of environmental stress on three Indo-Pacific corals were explored in this thesis. Temperature-irradiance stress treatments and temperature-nitrate stress treatments were examined on *S. pistillata*, *M. digitata* and *S. hystrix* to study the maximum quantum yield of dark-adaptated corals before stress, after stress and after 24-hr recovery period. The physical changes corals were also examined in both sets of experiments.

7.1.2 Temperature-irradiance stress on the corals

In the temperature-irradiance stress experiments, potential thermal tolerance of the coral species and changes in the coral physiology were studied to achieve the hypothesis. All the coral species after the stress treatments of high/ambient temperature and light levels had shown a significant

reduction in the maximum quantum yield of the chlorophyll fluorescence. There were changes of the PAM-fluorometer yield measurements between before and after stress conditions of the dark-adapted coral nubbins. The coral species had shown physical appearance changes and one mortality after the stress treatments. In temperature-irradiance stress experiments, each of the coral species had different response towards the stress treatments of high/ambient temperature and high/ambient light. This study only focused on the F_v/F_m values for the measurement of photosynthetic activity. Some treatments showed decline for some species, and showed a response, in terms of photoinhibition to combined stress of temperature and light. In chlorophyll fluorescence studies, photoinhibition is synonomous with interactions of high temperature and light on hard corals (the level of high light used in this study was 520 µmol quanta $m^{-2}s^{-1}$). Decreased values of F_v/F_m by fluorescence analysis revealed damage to the PSII reaction centre, which indicated changes in photosynthetic activity, leading to photoinhibition. *S. pistillata* and *M. digitata* only responded to high light levels alone, apart from the combinations of temperature and light stressors. *S. hystrix* was the only species that gave significant F_v/F_m values towards high light and high nitrate treatments and experienced bleaching and mortality after the experiment.

Bleaching is a general response of a coral towards stress, where they turn pale and lose their colour because of the loss of their symbiotic dinoflagellates. This study suggested that the response of the corals towards significant values of maximum quantum yield, their pale colouration and mortality rate are determining differential bleaching susceptibility for each of the coral species. It shows that *S. pistillata* and *M. digitata* might be tolerant to thermal stress and *S. hystrix,* susceptible towards thermal stress.

7.1.3 Temperature-nitrate stress on the corals

Scleractinian corals are able to live in nutrient-poor tropical waters due to symbiosis with photosynthetic dinoflagellates symbionts of *Symbiodinium* genus (Beraud et al., 2003). Nutrient concentrations in coral reef ecosystems are crucial for coral-zooxanthellae symbiosis, and can affect the function and composition of its photosynthetic process (Rodríguez-Román and Iglesias-Prieto, 2005). High nutrient levels in seawater encourage algal growth, cause low sunlight penetration and consequently out-compete corals for space and light, causing coral cover reduction and retard recovery (Harborne et al., 2000). *S. pistillata*, *M. digitata* and *S. hystrix* were exposed to four sets of treatment combinations of ambient/high temperature and ambient/high nitrate levels, to study the maximum quantum yield of dark-adaptation corals during before stress, after stress and after 24-hr recovery period. The coral's normal and pale colouration changes were also monitored throughout the experiments.

For temperature-nitrate stress experiments, nutrient-stress tolerance of the coral species and changes in the coral physiology were studied to address the hypothesis where a decrease in photosynthetic capacity will cause a change in the physical appearance of all coral species. All the coral species had shown significant effects of the maximum quantum yield measurements for the combined stress of the ambient/high levels of temperature/nitrates. During before and after stress conditions, the corals recorded a decreased value of the F_v/F_m for all the experiments. A few corals had shown changes in their colouration after the stress and after the 24-h recovery period, after high temperature and high nitrate levels experiment. This study suggested that high temperature is the main stressor to the coral species because the coral species had shown significant results only in combination stress of ambient/high nitrate with high temperature. *M. digitata* and *S. hystrix* might exhibit declines in zooxanthellae density as shown by their paling

colours after 5-h stress of the combined stressors of temperature and nitrate. In summary, this study of experimental stress of temperature and nitrate illustrated the impact on the coral-zooxanthellae symbioses by high temperature stressor alone, not by the nitrate enrichment stressor. This particular study also suggested that *S. pistillata*, the slower growing coral coral is more stress tolerant (nitrate specifically) than the fast growth corals; *M. digitata* and *S. hystrix,* based on their paling colour after stress experiments.

7.2 Thesis contributions

7.2.1 Corals' susceptibility

The stress experiments were only undertaken over short term exposures of 3-h and 5-h. This could be improved if the temperature-light and temperature-nitrate experiments were conducted for longer periods. More of the coral response will be seen if the stress experiments are for greater than 24 hours.

Coral bleaching and stress in this present study were measured under laboratory conditions, but could be verified by measurements *in situ* during a natural bleaching or stress events. A field study could use PAM fluorometer to measure effective quantum yield measurements, for light-adapted corals, and thus can add more reliable data for the normal conditions of the specific corals.

Temperature-light stress treatments from this study provided the measurements of dark-adapted maximum quantum yield for after stress and after a recovery period. The recorded quantum yield of the coral species from the study can produce an index of quantum yield of bleached corals of

temperature-light stressors. A decrease in photosynthetic capacity can cause photoinhibition, which is an early stress sign for coral bleaching. *S. hystrix* in this study was recorded bleached and mortality followed after the temperature-light treatments, which could provide the data for assessing the bleaching status of *S. hystrix* in a field or laboratory condition observation, in terms of maximum quantum yield measurement.

This study may also contribute reference data for a coral susceptibility towards bleaching. *S. hystrix* was shown to be highly susceptible to bleaching while *S. pistillata* and *M. digitata* can be recorded as thermal-tolerant corals because they survived the temperature-light stress experiments in this study. If there are any monitoring activities of bleaching events in Malaysia, a park manager or a researcher can survey *S. hystrix*, for any early sign of coral community stress.

Temperature-light stress levels from this study could be a reference to predict stress conditions for these specific corals in the future studies. A high temperature of 30°C and high light levels of 520 µmol quanta $m^{-2}s^{-1}$ reduced the maximum quantum yield measurements of *S. pistillata* by 20.7%, *M. digitata* by 18.5% and *S. hystrix* by 19%. The specific stress levels were also confirmed to be the cause of *S. hystrix* bleached and had mortality in the study. Therefore, combinations or stand-alone stress levels of 30 °C and 520 µmol quanta $m^{-2}s^{-1}$ irradiance can be a baseline data for the prediction of coral stress and bleaching.

Table 7.1 The reduction of maximum quantum yield measurements for three coral species for three treatments after stress and after 24h post-stress.

Corals	Treatments	After stress	After post-stress
Ambient temperature and high light	*S. pistillata*	22.77%	3.08%
	M. digitata	44.72%	25.76%
	S. hystrix	27.84%	2.64%
High temperature and ambient light	*S. pistillata*	10.73%	0.38%
	M. digitata	19.76%	12.27%
	S. hystrix	5.42%	3.06%
High temperature and high light	*S. pistillata*	20.70%	13.48%
	M. digitata	18.45%	13.16%
	S. hystrix	18.97%	8%

In the second part of the present study, the data of temperature-nitrate stress treatments towards the specific coral species can help the future researchers and marine park management in Malaysia to predict and study the nitrate-stressed corals. This study identified the maximum quantum yield measurements of dark-adapted specific coral species for nitrate-stress conditions and showed that low nitrate does not have any effect on the corals. It can help in understanding the coral conditions in the higher nitrate condition area, in terms of decreased photosynthetic quantum yield data. Even though the corals in the affected area may not show any visible sign of stress conditions, a PAM fluorometer may be able to measure it. A normal looking coral in areas of untreated sewage coastal development and sedimentation, may show early signs of stress by decreased maximum quantum yield measurement. The authorities can do the survey monitoring on the corals health before, during and after a bleaching event to record the normal

readings and stressed readings of coral's yield. Maximum quantum yield of a coral is between 0.5-0.8 value of F_v/F_m based on species. With the guideline, any readings below than that can identify non-healthy corals. This knowledge can be used to build a coral survey database based on the maximum quantum yield measurement, supporting actions to be taken by the MPA's managers. Thus, the MPA management can help to minimise coral stress and damage.

High temperatures of 30ºC and high nitrate levels of 15 µM NO_3^- reduced maximum quantum yield measurements of *S. pistillata, M. digitata* and *S. hystrix* after the 5-h stress treatments. Therefore, this reference can predict any nitrate-stress of the specific corals in other future studies. Information on stress-tolerant species such as *S. pistillata* can also be a reference for other researchers regarding temperature-nitrate stress experiments or natural events. In this study, *S. pistillata* was a stress-tolerant species towards temperature-nitrate stress because it can survive throughout the experiments, in terms of the coral physiology.

7.2.2 Short term shock experiments

This study can be a contribution to the field to examine the usefulness of short term shock experiments towards coral bleaching. To understand the mechanisms of coral bleaching, it is also important to determine the role and time-frame of the heat stress and light stress act induce a bleaching event (Downs et al., 2013). Coral bleaching had been associated with short-term temperature increases, ranging from few hours to few days (Middlebrook et al., 2008).

Short term exposure can determine the initial responses of zooxanthellae towards bleaching conditions (significantly reduced zooxanthellae density or chlorophyll levels) within 24-48 hours observation. Dinoflagellate symbionts in *Acropora aspera* were exposed to 31ºC for 48

hours during pre-stress treatment had a great reduction in their photosynthetic efficiency (Middlebrook et al., 2008). Referring to Gibbin et al., (2018), short-term acclimation in two days had reduced heat stress-related protein damage in *Acropora nana*. Downs et al. (2013) 48 hours of heat stress in 32ºC had induced decomposition of thylakoid structures which can lead to photo-oxidative stress in *P. damicornis.*

In this study, for 3h and 5h of coral stressors had reduced the maximum quantum yield of three species corals in a lab-controlled conditions. It is suggested this outcome can be compared to *in situ* field assessment to discovery the coral's bleaching vulnerability in short-term heat exposed. The responses might be one of the multiple ways of coral acclimatization for different stress within different timeframes. Thus, a broader study can be done towards effects of short term shock experiments onto more coral species to understand the thermal threshold of corals. A short-term shock experiments can be the initial coral bleaching responses which are able to predict the coral's bleaching susceptibility and severity.

7.3 Future work

This present study is only concerned with measuring stress in corals by maximum quantum yield measurements using PAM-fluorometer, and there are many more factors than can be considered to strengthen the study. The damage to the zooxanthellae photosynthetic capability can be further examined in terms of their morphology; maybe by looking into chlorophyll *a* concentration and the zooxanthellae density, and this can be compared to the maximum quantum yield data. The coral physiological response towards stress can be further understood, beyond observing coral paling colouration.

This present study can be enhanced by studying the response of corals towards combinations of stressors of temperature, light and nitrate, which mimic the environmental disturbance *in situ*. Stressors could be studied by combinations (i.e; temperature and nutrient stress, light and nutrient stress, sedimentation and temperature stress) or stand-alone factors (i.e; temperature alone, ocean acidification alone, sedimentation alone to understand the interaction and effects of environmental disturbance and climate change (high temperature and high light).

Laboratory condition effects can be used to develop the feedback monitoring loop for an action plan to manage the coral stress in natural environment. The loop is based on the maximum quantum yield measurement on normal corals and non-healthy corals in the field. The yield measurement can be compared during before and after a bleaching event and routine survey must be done by the authorities to achieve the action plan. This monitoring management can determine the coral's health in the field, and if the corals is not healthy, it can be determined whether their deteriorating health is affected by the anthropogenic impact or the global warming or both. Besides the coral survey itself, other side-effect impact monitoring such as the level of nutrient status or the sedimentation levels must be done to study the impact onto the coral's health.

References

Downs, C.A., McDougall, K.E., Woodley, C.M., Fauth, J.E., Richmond, R.H., Kushmaro, A., Gibb, S.W., Loya, Y., Ostrander, G.K. and Kramarsky-Winter, E., 2013. Heat-stress and light-stress induce different cellular pathologies in the symbiotic dinoflagellate during coral bleaching. PLoS One, 8(12), p.e 77173.

Gibbin, E.M., Krueger, T., Putnam, H.M., Barott, K.L., Bodin, J., Gates, R.D. and Meibom, A., 2018. Short-Term Thermal Acclimation Modifies the Metabolic Condition of the Coral Holobiont. Frontiers in Marine Science, 5, p.10.

Langenheim J. 2016. Malaysia establishes a 1-million-hectare marine park. www.theguardian.com/world/blog. 30th May 2016.

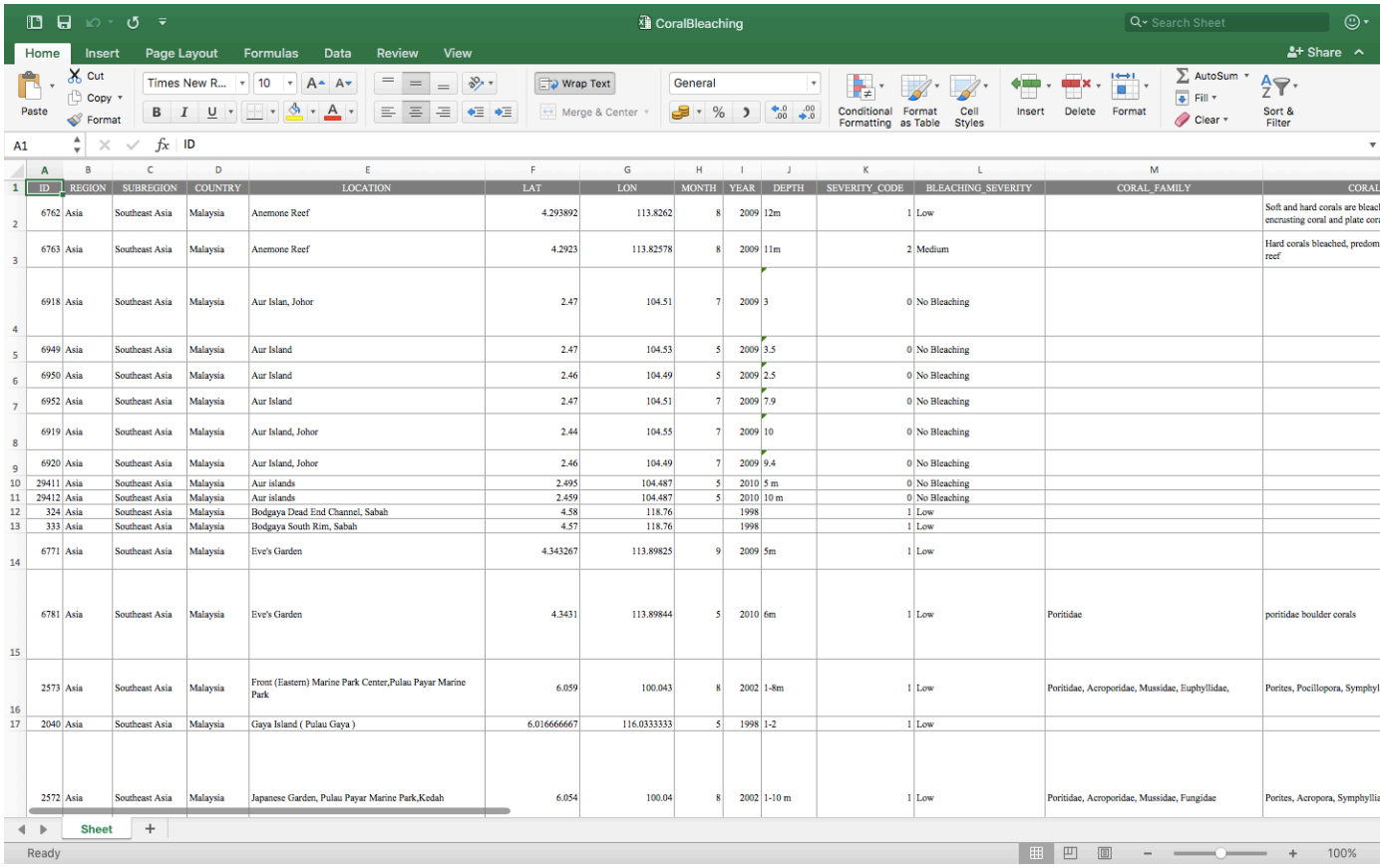
Middlebrook, R., Hoegh-Guldberg, O. and Leggat, W., 2008. The effect of thermal history on the susceptibility of reef-building corals to thermal stress. *Journal of Experimental Biology*, *211*(7), pp.1050-1056.

Reef Check. 2012. Status of Coral Reefs in Malaysia.

Reef Check. 2015. Status of Coral Reefs in Malaysia.

West, J. M. and Salm, R. V. 2003. Resistance and Resilience to Coral Bleaching: Implications for Coral Reef Conservation and Management. *Conservation Biology* **17: 956–967.**

Appendix A



CoralBleaching

ID	REGION	SUBREGION	COUNTRY	LOCATION	LAT	LON	MONTH	YEAR	DEPTH	SEVERITY_CODE	BLEACHING_SEVERITY	CORAL_FAMILY	CORAL
6762	Asia	Southeast Asia	Malaysia	Anemone Reef	4.293892	113.8262	8	2009	12m	1	Low		Soft and hard corals are bleacl encrusting coral and plate cor
6763	Asia	Southeast Asia	Malaysia	Anemone Reef	4.2923	113.82578	8	2009	11m	2	Medium		Hard corals bleached, predom reef
6918	Asia	Southeast Asia	Malaysia	Aur Islan, Johor	2.47	104.51	7	2009	3	0	No Bleaching		
6949	Asia	Southeast Asia	Malaysia	Aur Island	2.47	104.53	5	2009	3.5	0	No Bleaching		
6950	Asia	Southeast Asia	Malaysia	Aur Island	2.46	104.49	5	2009	2.5	0	No Bleaching		
6952	Asia	Southeast Asia	Malaysia	Aur Island	2.47	104.51	7	2009	7.9	0	No Bleaching		
6919	Asia	Southeast Asia	Malaysia	Aur Island, Johor	2.44	104.55	7	2009	10	0	No Bleaching		
6920	Asia	Southeast Asia	Malaysia	Aur Island, Johor	2.46	104.49	7	2009	9.4	0	No Bleaching		
29411	Asia	Southeast Asia	Malaysia	Aur islands	2.495	104.487	5	2010	5 m	0	No Bleaching		
29412	Asia	Southeast Asia	Malaysia	Aur islands	2.459	104.487	5	2010	10 m	0	No Bleaching		
324	Asia	Southeast Asia	Malaysia	Bodgaya Dead End Channel, Sabah	4.58	118.76		1998		1	Low		
333	Asia	Southeast Asia	Malaysia	Bodgaya South Rim, Sabah	4.57	118.76		1998		1	Low		
6771	Asia	Southeast Asia	Malaysia	Eve's Garden	4.343267	113.89825	9	2009	5m	1	Low		
6781	Asia	Southeast Asia	Malaysia	Eve's Garden	4.3431	113.89844	5	2010	6m	1	Low	Poritidae	poritidae boulder corals
2573	Asia	Southeast Asia	Malaysia	Front (Eastern) Marine Park Center,Pulau Payar Marine Park	6.059	100.043	8	2002	1-8m	1	Low	Poritidae, Acroporidae, Mussidae, Euphyllidae,	Porites, Pocillopora, Symphyl
2040	Asia	Southeast Asia	Malaysia	Gaya Island (Pulau Gaya)	6.016666667	116.0333333	5	1998	1-2	1	Low		
2572	Asia	Southeast Asia	Malaysia	Japanese Garden, Pulau Payar Marine Park,Kedah	6.054	100.04	8	2002	1-10 m	1	Low	Poritidae, Acroporidae, Mussidae, Fungidae	Porites, Acropora, Symphyllia

Figure A.1: Screenshot of raw data of coral bleaching survey from Reef Check Malaysia. Records of coral bleaching event since 1998 until 2010 were organised based on locations in alphabetically order, the latitude and longitude, water depth, bleaching severity and the bleached coral's family/species.

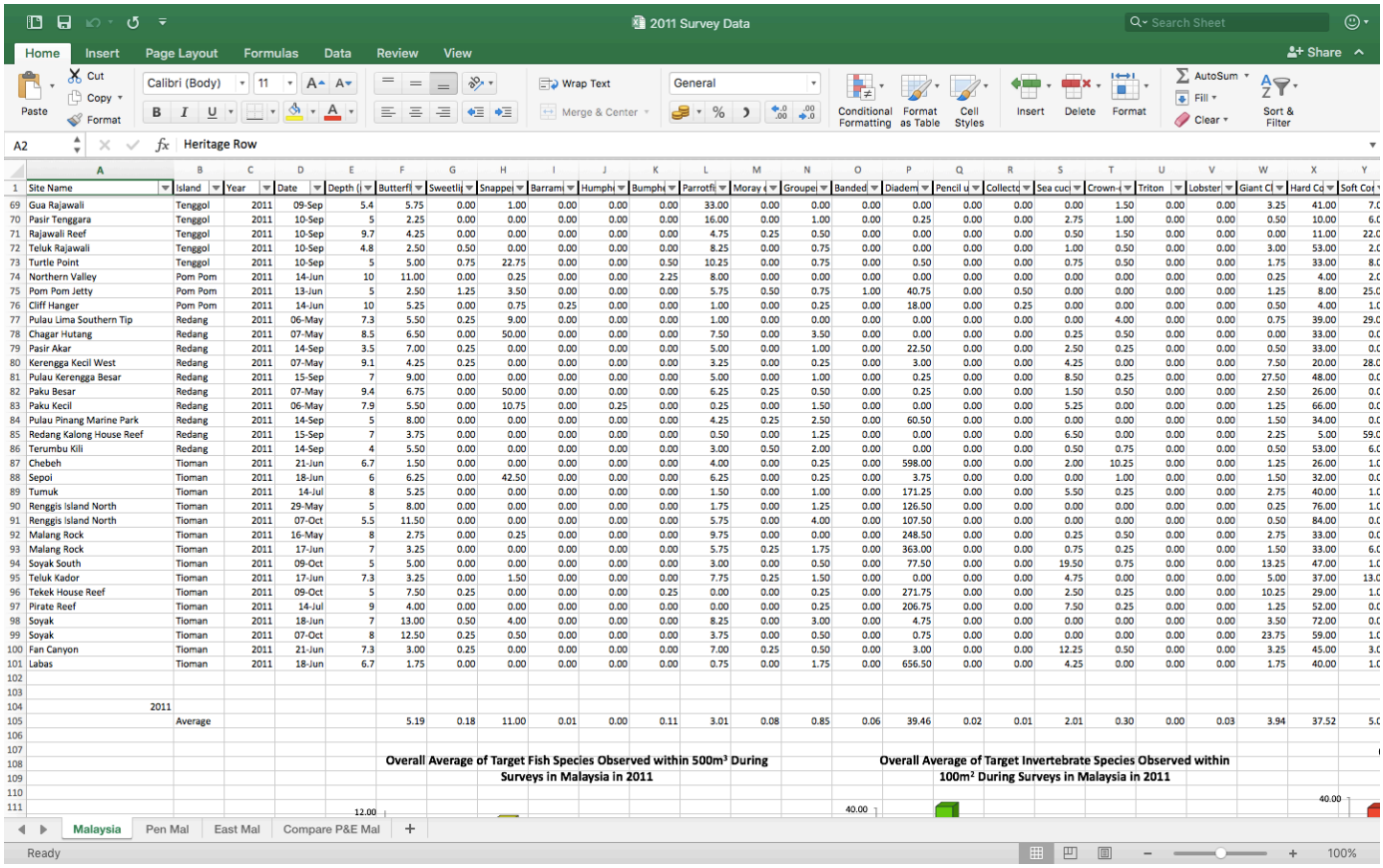


Figure A.2: Screenshot of raw data Reef Check Survey 2011

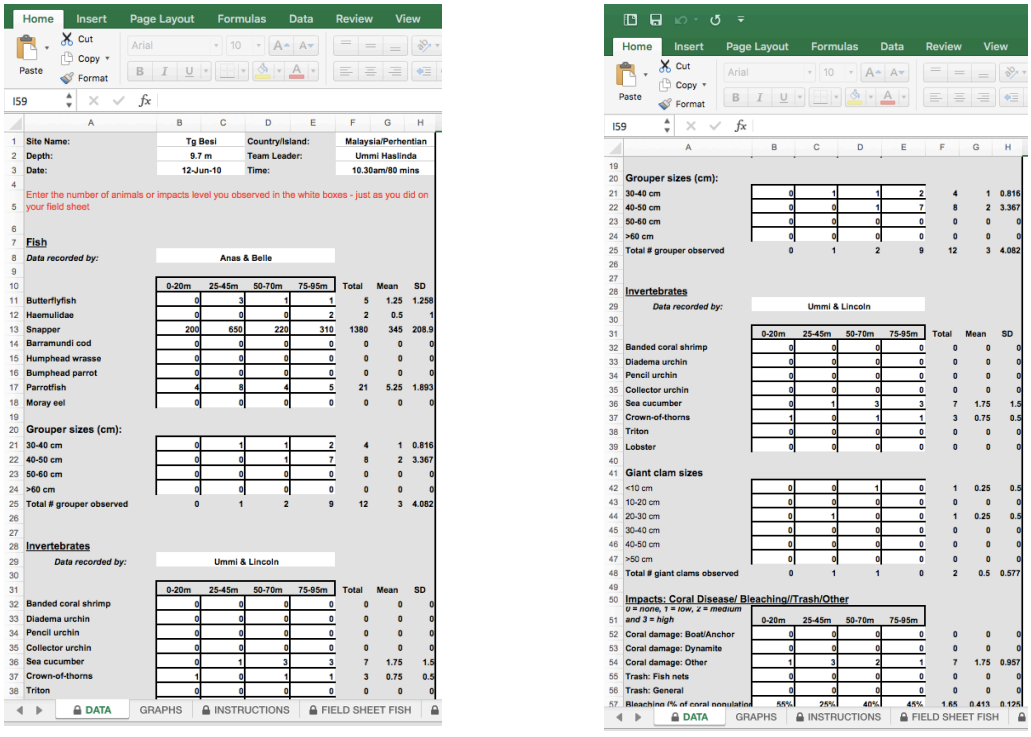
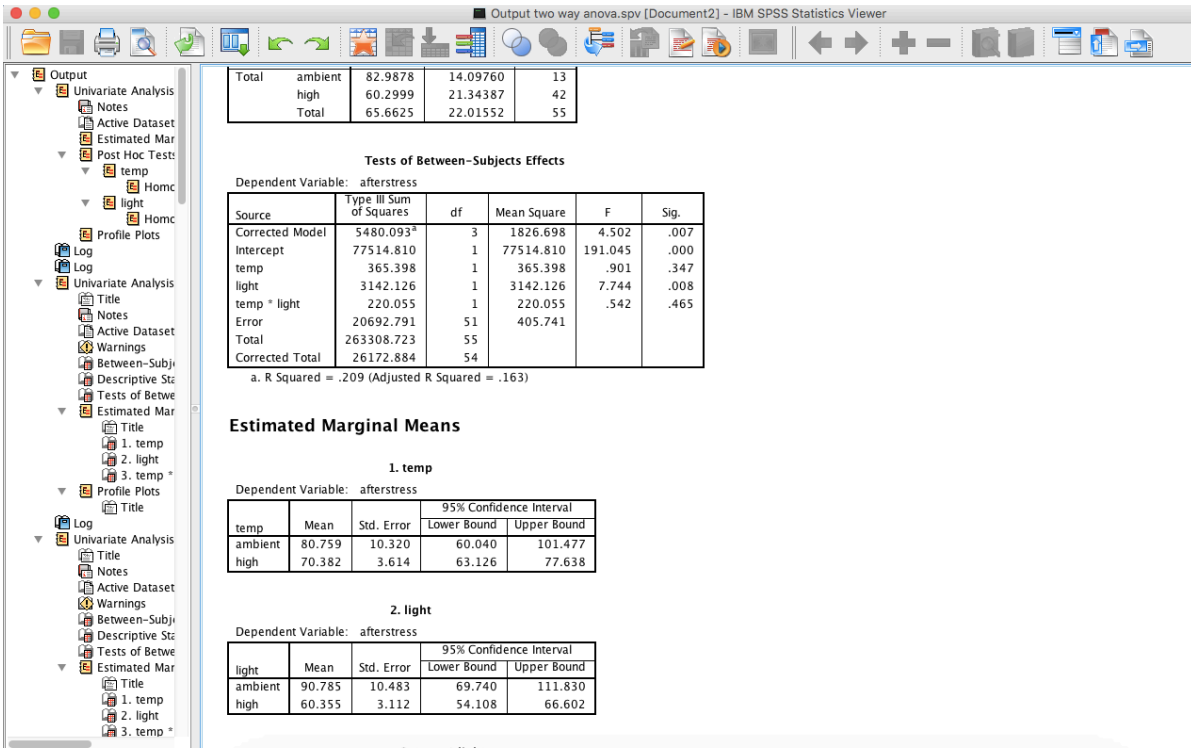


Figure A.3: Screenshot of one of the raw data Reef Check Survey 2010 (Site location:

Tanjung Besi, Perhentian island)

Appendix B



Total	ambient	82.9878	14.09760	13
	high	60.2999	21.34387	42
	Total	65.6625	22.01552	55

Tests of Between-Subjects Effects

Dependent Variable: afterstress

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5480.093[a]	3	1826.698	4.502	.007
Intercept	77514.810	1	77514.810	191.045	.000
temp	365.398	1	365.398	.901	.347
light	3142.126	1	3142.126	7.744	.008
temp * light	220.055	1	220.055	.542	.465
Error	20692.791	51	405.741		
Total	263308.723	55			
Corrected Total	26172.884	54			

a. R Squared = .209 (Adjusted R Squared = .163)

Estimated Marginal Means

1. temp

Dependent Variable: afterstress

temp	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
ambient	80.759	10.320	60.040	101.477
high	70.382	3.614	63.126	77.638

2. light

Dependent Variable: afterstress

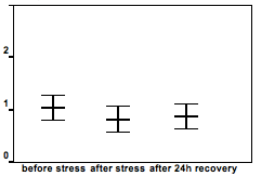
light	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
ambient	90.785	10.483	69.740	111.830
high	60.355	3.112	54.108	66.602

One way ANOVA

Temperature and light stress experiment: **High temperature and high light stress treatment, 3 hours**

Coral: *S. pistillata*

source	df	SS	MS	F	P-value
treatments	2	0.213	0.107	7.5964	0.0008
error	123	1.728	0.014		
total	125	1.941			



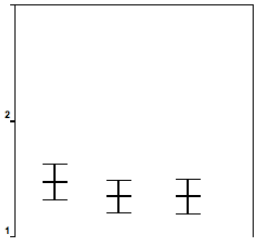
ANOVA

One-way completely randomized

High temperature and high light stress on M. digitata

	before stress	after stress	after 24h recovery
1	0.473	0.498	0.385
2	0.401	0.53	0.372
3	0.397	0.474	0.301
4	0.281	0.496	0.505
5	0.5	0.493	0.013
6	0.469	0.515	0.387
7	0.362	0.484	0.224
8	0.259	0.483	0.323
9	0.72	0.474	0.464
10	0.464	0.482	0.179
11	0.683	0.077	0.342
12	0.627	0.312	0.378
13	0.247	0.426	0.376
14	0.941	0.337	0.378
15	0.625	0.269	0.376
16	0.53	0.243	0.182
17	0.571	0.33	0.338
18	0.431	0.272	0.372
19	0.147	0.248	0.288
20	0.377	0.268	0.211
21	0.298	0.234	0.731
22	0.362	0.071	0.175
23	0.538	0.183	0.43
24	0.361	0.303	0.242
25	0.409	0.161	0.219
26	0.752	0.196	0.246
27	0.54	0.234	0.324
28	0.659	0.318	0.13
29	0.563	0.234	0.8
30	0.481	0.194	0.445
31	0.429	0.328	0.281
32	0.469	0.579	0.494
33	0.445	0.63	0.528
n	33	33	33
X	0.479	0.345	0.347
s	0.163	0.147	0.158
X_{ave}	0.390		

source	df	SS	MS	F	P-value
treatments	2	0.392	0.196	8.0381	0.0006
error	96	2.340	0.024		
total	98	2.731			



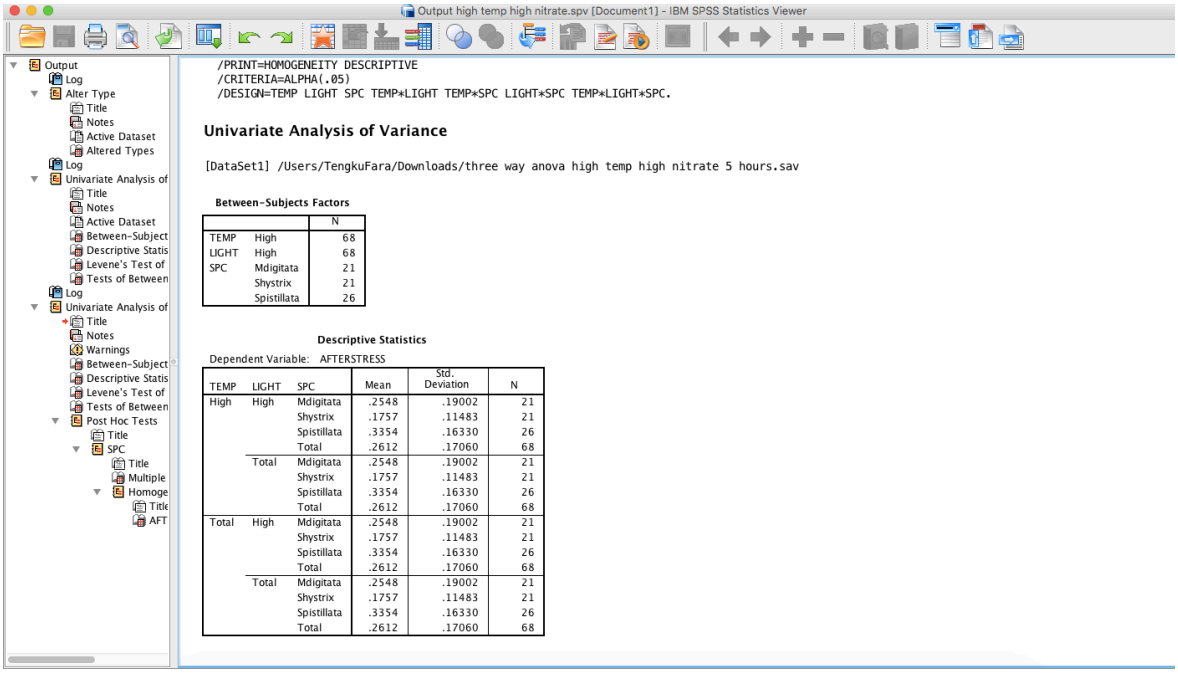


Figure B.1: Screenshot of SPSS three-way ANOVA report

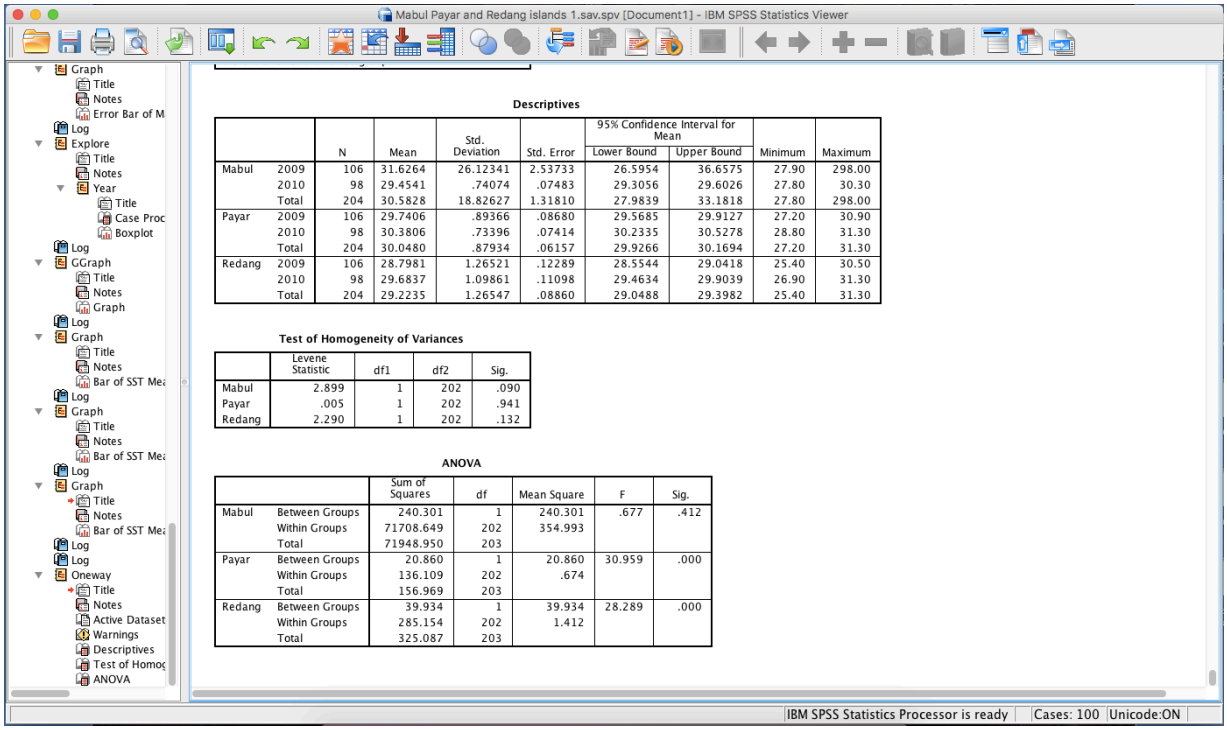


Figure B.2: Screenshot of SPSS one-way ANOVA report

Appendix C

Table C1: Multiple comparison ANOVA for F_v/F_m mean values of *S. pistillata*, *M. digitata* and *S. hystrix* during before stress, after 5-hr stress and after 24-hr recovery period for ambient temperature and high nitrate stress treatments.

Dependent Variable	(I) SPC	(J) SPC	Std. Error	Sig.
beforestress	M. digitata	S. hystrix	.05739	.961
		S. pistillata	.05334	.002
	S. hystrix	M. digitata	.05739	.961
		S. pistillata	.05607	.008
	S. pistillata	M. digitata	.05334	.002
		S. hystrix	.05607	.008
afterstress	M. digitata	S. hystrix	.04733	.518
		S. pistillata	.04398	.000
	S. hystrix	M. digitata	.04733	.518
		S. pistillata	.04624	.011
	S. pistillata	M. digitata	.04398	.000
		S. hystrix	.04624	.011
afterrecovery	M. digitata	S. hystrix	.06704	.933
		S. pistillata	.06230	.243
	S. hystrix	M. digitata	.06704	.933
		S. pistillata	.06550	.145
	S. pistillata	M. digitata	.06230	.243
		S. hystrix	.06550	.145

Appendix D

Table D.1: Peninsular Malaysia survey sites in Reef Check survey

Site Name	Island / Region	Site Name	Island / Region
Batu Layar	Perhentian	Pasir Tenggara	Bidong/Yu
Batu Nisan	Perhentian	Pulau Karah	Bidong/Yu
Batu Tabir	Perhentian	Pulau Tengkorak	Bidong/Yu
D'Lagoon	Perhentian	Pulau Yu Besar	Bidong/Yu
Lighthouse	Perhentian	Pulau Yu Kecil	Bidong/Yu
Pulau Rawa	Perhentian	Fresh Water Bay	Tenggol
Sea Bell	Perhentian	Gua Rajawali	Tenggol
Sharkpoint	Perhentian	Pasir Tenggara	Tenggol
Tanjung Besi	Perhentian	Rajawali Reef	Tenggol
Tiga Ruang	Perhentian	Teluk Rajawali	Tenggol
Tukas Laut	Perhentian	Turtle Point	Tenggol
Pulau Lima Southern Tip	Redang	Chebeh	Tioman
Chagar Hutang	Redang	Sepoi	Tioman
Pasir Akar	Redang	Tumuk	Tioman
Kerengga Kecil West	Redang	Renggis Island North	Tioman

Pulau Kerengga Besar	Redang	Renggis Island North	Tioman
Paku Besar	Redang	Malang Rock	Tioman
Paku Kecil	Redang	Malang Rock	Tioman
Pulau Pinang Marine Park	Redang	Soyak South	Tioman
Redang Kalong House Reef	Redang	Teluk Kador	Tioman
Terumbu Kili	Redang	Tekek House Reef	Tioman
Coral Garden 1	Kapas	Pirate Reef	Tioman
Coral Garden 3	Kapas	Soyak	Tioman
Silent Reef	Kapas	Soyak	Tioman
Teluk Jawa	Kapas	Fan Canyon	Tioman
Heritage Row	Bidong/Yu	Labas	Tioman

Table D.2: East Malaysia survey sites in Reef Check survey

Site Name	Island / Region	Site Name	Island / Region
Batu Penyu	Talang-Satang	Edwin Rock	Lankayan
Talang Besar East	Talang-Satang	Moray	Lankayan
Talang Besar West	Talang-Satang	Veron Shallow	Lankayan
Anemone North	Miri	Ken's Rock	Lankayan
Sunday Reef	Miri	Reef 77	Lankayan
Batu Batik	Miri	Ikok Rock	Lankayan
Beting Niah North	Miri	Malu Malu	Lankayan
Beting Niah South	Miri	Twin Rock	Lankayan
Eve's Garden	Miri	Cahaya Way, Bohayan Island	Mataking/Pom Pom
Siwa 4	Miri	Timba Timba, Sting Ray City	Mataking/Pom Pom
Froggie Fort	Lankayan	Pandanan Bay, Pandanan Island	Mataking/Pom Pom
G. Kolam	Lankayan	Coral Garden Mataking	Mataking/Pom Pom
Katching Star (new)	Lankayan	Mataking House Reef	Mataking/Pom Pom

Bimbo Rock (new)	Lankayan	Sweetlips Rock Mataking	Mataking/Pom Pom
SSR	Lankayan	Northern Valley	Mataking/Pom Pom
Reef 38	Lankayan	Pom Pom Jetty	Mataking/Pom Pom
Mels Rock (new)	Lankayan	Cliff Hanger	Mataking/Pom Pom
Jawfish	Lankayan	Scuba Junkie House Reef M	Mabul
Sand Bar South	Lankayan	Scuba Junkie House Reef S	Mabul
Zorro	Lankayan	Paradise 2 M	Mabul
Sand Bar North	Lankayan	Paradise 2 S	Mabul
Goby Rock	Lankayan	Eel Garden	Mabul
Lycia Garden	Lankayan	Mandarin Valley	Mabul
Pegaso (new)	Lankayan	Panglima Reef	Mabul

Mabul Island

BYYY	BM	BD	BH	EYYY	EM	ED	EH	SST	SSTANOM	HOTSPOT	DHW	Lat	Lon	Reef Name
2009	01	05	05	2009	01	08	03	29.30	1.15	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	01	08	07	2009	01	12	10	29.30	1.18	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	01	12	10	2009	01	15	04	29.30	1.20	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	01	15	04	2009	01	19	03	29.30	1.25	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	01	19	03	2009	01	22	04	28.20	0.19	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	01	22	04	2009	01	26	03	28.40	0.45	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	01	26	03	2009	01	29	03	28.40	0.49	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	01	29	03	2009	02	02	04	28.10	0.24	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	02	02	04	2009	02	05	03	28.10	0.28	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	02	05	03	2009	02	09	04	27.90	0.13	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	02	09	04	2009	02	12	04	28.50	0.77	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	02	12	04	2009	02	16	03	28.10	0.35	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	02	16	03	2009	02	19	05	28.50	0.68	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	02	19	05	2009	02	23	04	29.00	1.08	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	02	23	04	2009	02	26	03	29.00	1.01	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	02	26	03	2009	03	02	05	28.70	0.61	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	02	26	03	2009	03	02	05	28.70	0.61	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	03	02	05	2009	03	05	03	29.00	0.84	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	03	05	03	2009	03	09	03	29.00	0.74	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	03	09	03	2009	03	12	05	28.80	0.47	0.00	0.00	4.5	119.0	Mabul Island, Malaysia
2009	03	12	05	2009	03	16	03	29.20	0.78	0.00	0.00	4.5	119.0	Mabul Island, Malaysia

Figure D.1: Part of Virtual stations NOAA data for Mabul, Payar and Redang islands from 2009 to 2011.