EFFECTS OF TEMPERATURE STRESS ON *Symbiodinium* spp. FROM SELECTED SCLERACTINIANS

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EFFECTS OF TEMPERATURE STRESS ON *Symbiodinium* spp. FROM SELECTED SCLERACTINIANS

by

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Nadthikphorn D/O Kamphol

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ABSTRAK

Symbiodinium dijelaskan sebagai endo-symbion yang berbentuk kokoid dan berwarna kuning coklat, hidup dalam tisu karang serta menyediakan produk fotosintesis yang diperlukan untuk karang. Walau bagaimanapun, dalam keadaan yang tertekan seperti perubahan suhu, hubungan simbiotik antara *Symbiodinium* dan karang akan terjejas dan mengakibatkan pemutihan karang. Oleh itu, kajian ini dijalankan untuk merekod spesis karang yang ditemui di Pulau Songsong, Pulau Kendi dan Pulau Redang, serta untuk mengkaji kesan perubahan suhu terhadap kepadatan dan kesihatan sel *Symbiodinium* dalam kalangan spesis karang. Sepuluh famili dengan jumlah 21 spesies karang telah direkodkan. *Diving-Pulse Amplitude Modulation* (PAM) digunakan untuk menentukan nilai maksimum kuantum (beradaptasi gelap) (F_v/F_m), yang merupakan penunjuk untuk menilai status kesihatan semasa karang. Nilai *in-situ* F_v/F_m *Porites somaliensis*, *Porites lutea*, *Goniopora cellulosa, Turbinaria mesenterina, Oulastrea crispata, Cyphastrea chalcidicum*, *Goniastrea retiformis* dan *Pavona danai* menunjukkan karang ini sihat dan telah beradaptasi dengan keadaan maksimum cahaya bawah (600μmol quanta $m^{-2}s^{-1}$) pada hari persampelan. Koloni karang berliku dan bersaiz besar seperti *Coelastrea aspera* dan *Goniopora cellulosa* dirakam tinggi dalam kepadatan zooxanthellae, $0.258\pm0.134x10^{-5}cm^{-2}$ dan $0.22\pm 0.229x10^{-5}cm^{-2}$, di dalam 40-60 mg/L jumlah pepejal terampai di kawasan air keruh. Selain itu, eksperimen tekanan haba telah dijalankan untuk mengkaji bagaimana zooxanthellae berkembang subur dalam suhu yang tinggi.

Hasil kajian menunjukkan bahawa setiap karang mengeluarkan dua bentuk morfologi *Symbiodinium*, sel yang sihat dan terdegradasi, di mana nisbah bentuk ini adalah berbeza bergantung pada suhu yang didedahkan. Karang mengeluarkan sel-sel *Symbiodinium* yang sihat dan terdegradasi dalam nisbah 4: 6 apabila terdedah kepada 30°C-33°C. Sebaliknya, apabila suhu meningkat kepada 33°C-37°C, kadar pengeluaran selnya meningkat secara drastik, kecuali *Goniopora cellulosa*, dan hampir 80% daripada sel yang dikeluarkan adalah sel *Symbiodinium* yang sihat. Hasil ini menunjukkan bahawa dalam keadaan tidak tertekan, karang mengawal kepadatan *Symbiodinium* dengan mengeluarkan sel yang terdegradasi. Fungsi ini akan terjejas apabila terdedah kepada suhu yang tinggi, menyebabkan kehilangan sel *Symbiodinium* yang sihat tetapi fotosintetiknya mengalami kerosakan di mana nilai F_v/F_m semakin berkurangan. Hasil kajian menunjukkan *Porites lutea*, karang berliku dan bersaiz besar adalah spesies karang yang paling tahan terhadap air yang keruh dan suhu yang tinggi. Kesimpulannya, karang mampu beradaptasi dalam keadaan tertekan melalui tindakan mekanisma foto-protektif, seperti peningkatan atau pengurangan pigmen foto-protektifnya untuk memaksimakan penangkapan cahaya atau mengawal kepadatan *Symbiodinium* hidup dalam polip karang.

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ABSTRACT

Symbiodinium is described as a coccoid yellow-brown endo-symbiont, which inhabits coral tissues and provides photosynthetic products necessary for corals. However, under stressful conditions such as temperature change, the symbiotic relationship between *Symbiodinium* and corals may collapse and result in coral bleaching. Hence, this study was conducted to record coral species found in Pulau Songsong, Pulau Kendi and Pulau Redang, and to investigate the effects of temperature increased on the *Symbiodinium* cell density and health among coral species. Ten families with a total of 21 coral species were recorded. Diving-Pulse Amplitude Modulation (PAM) was used to determine the maximum quantum yield values (dark-adapted) (F_v/F_m), which is an indicator that helps to assess the current health status of corals. The *in-situ* F_v/F_m value of *Porites somaliensis, Porites lutea*, *Goniopora cellulosa, Turbinaria mesenterina, Oulastrea crispata, Cyphastrea chalcidicum*, *Goniastrea retiformis* and *Pavona danai* indicated that these corals were healthy and had adapted to maximum bottom light condition (600µmol quanta $m^{-2}s^{-1}$) during sampling day. Massive meandroid coral colonies like *Coelastrea aspera* and *Goniopora cellulosa* recorded high in its zooxanthellae density, $0.258\pm0.134x10^{-5}cm^{-2,}$ and $0.224\pm0.229x10^{-5}cm^{-2}$, respectively, in 40-60mg/L total suspended solids (TSS) of turbid water area. Moreover, thermal stress experiments were conducted to investigate how the zooxanthellae thrive at 31˚C to 37˚C of water temperature. Results obtained indicated that every coral expelled two forms of

Symbiodinium morphology, which were healthy and degraded cells, and the ratios of these forms differ depending on the exposed temperature. Corals expelled healthy and degraded *Symbiodinium* cells in the ratio 4:6 when exposed to 30˚C -33˚C. In contrast, when the temperature was increased to 33˚C-37˚C, the expulsion rate drastically increased, except *Goniopora cellulosa*, where almost 80% of the expelled populations exhibited healthy *Symbiodinium* cells. This result indicated that under non-stress conditions, the corals regulate *Symbiodinium* density by expelling degraded cells. This function will break down under high temperature, leading to extensive loss of healthy but photosynthetically damaged *Symbiodinium* cells as their F_v/F_m value was gradually decreased. It was found that *Porites lutea*, the massive meandroid coral is the most resistant coral species towards the turbid water and high temperature. In conclusion, corals could adapt to stress condition through the action of photoprotective mechanisms such as increasing or decreasing its photo-protective pigments to gain maximum light capture or controlling its *Symbiodinium* density inhibit within coral polyps.

CHAPTER 1.0

GENERAL INTRODUCTION

1.1 Coral and Its Symbionts

A coral polyp is a tube-like animal with a central mouth on the top of polyps, which also performs as the host of millions of microscopic algae called “zooxanthellae”. These zooxanthellae also contribute to the growth of the corals. Zooxanthellae accelerate the coral's calcification process and provide over 98% of the polyp's nutritional requirements such as glucose, glycerol and amino acids. Also, zooxanthellae cell density within coral polyps multiplied by utilizing the coral's nitrogenous waste and carbon dioxide. This symbiotic relationship is mutually beneficial to both coral and its symbionts (Muller-Parker & D’Elia, 2015).

Zooxanthellae are like all photosynthetic organisms. They utilize the energy from the sun to convert water and carbon dioxide into oxygen, sugars, and starches. Hence, sunlight is needed for the survival of reef-building (zooxanthellae species) types. This answers why most corals are found growing well in shallow waters (less than 30 meters) as compared to deep waters. On the other hand, for the non-reef-building corals (azooxanthellae species), they are not affected by the sunlight. Therefore, these corals could be found thriving thousand meters deep into the water (Stanley, 2003).

Zooxanthellae have the capability to transform the corals into a variety of colours, such as brown, green, or orange. Thus, when corals are exposed to a stressful condition such as increasing water temperature, the colour of corals will turn pale and gradually become white because pigmentation in hermatypic corals is generally derived from zooxanthellae, not pigment in the coral tissue. The loss of zooxanthellae causes the coral tissues to become transparent, allowing its white calcium carbonate skeleton

to be readily viewed through its transparent tissue and causing corals to appear in white which known as “coral bleaching phenomenon”.

Bleaching event is considered as a stress symptom of corals and its zooxanthellae which may be induced by a variety of factors, including fluctuation of seawater temperature, underwater light intensity, salinity, and pollution. Metabolic rates of corals and its symbionts could be affected by seawater temperature, which depends on how photosynthesis and respiration of both zooxanthellae and coral hosts respond to the changes in temperature. In addition, Fitt *et.al.* (2000) explained that steady increases in seawater temperature will cause an increase in corals and zooxanthellae respiratory metabolism which decrease the energy reserves in coral tissue. Therefore, when seawater temperature is higher than its average mean, it would induce rapid declination in zooxanthellae density within coral polyps, leading to coral discolouration.

1.2 Thesis Outline

Increasing of water sediment delivery to the coastal ocean could results in chronic sediment stress to coral reefs (Jokiel et al. 2004). However, in this study, the coral species collected could flourish in naturally turbid environments, especially in Pulau Songsong and Pulau Kendi. Meanwhile, the zooxanthellae able to cope with significant daily variation in light intensities due to turbidity. Therefore, this study was undertaken to understand how these scleractinians could adapt to turbid water, especially corals from Pulau Songong and Pulau Kendi. These coral species were identified to lowest taxonomic levels before proceed to investigate in details on the relationship of environmental parameters towards the health of coral’s symbionts. The culture of extracted *Symbiodinium* cells was conducted to get a better understanding of morphological of healthy and degrade *Symbiodinium* cells.

It is generally known that corals have a symbiosis relationship with dinoflagellates known as zooxanthellae. Hence, this study focuses more on the relationship and how environmental factors such as limitation of light penetration in water and increase of water temperature could alter the relationship between zooxanthellae and coral species. This study emphasizes the symbiotic relationship of coral and its symbionts, where it is divided into four different parts. They are (i) Identification of corals from Pulau Songsong, Pulau Kendi, and Pulau Redang; (ii) The coral colonies and its symbionts relationship; (iii) Survival rate of coral symbionts in different media; and (iv) Effect of thermal stress on the health of coral symbionts.

1.3 Objectives

The objectives of this study are:

1. To identify and record coral species which inhabit Pulau Songsong, Pulau Kendi, and Pulau Redang to the lowest taxonomy level.
2. To investigate the significance of seawater physical parameters such as light intensity, total suspended solids, water temperature, dissolved oxygen content, pH, and salinity towards the *Symbiodinium* cell density and its photosynthetic pigment concentration from different coral hosts.
3. To examine the significant effect of increasing water temperature towards *Symbiodinium* cells in 48 hours incubations and 24 hours incubation.

1.4 Hypotheses

The null hypotheses (H_0) of this study are:

1. No divergent in biomass composition of coral symbionts among the coral species in stressful seawater condition, especially light limited and high water temperature conditions.
2. The increment of water temperature cause no significant effect on the photosynthetic activity and biomass of *Symbiodinium* among different coral colonies, either incubate in 48 hours nor 24 hours period.

The hypotheses (H_α) of this study are:

1. The different coral host could alter its zooxanthellae density and photosynthetic pigment concentration within the stressful seawater condition, especially light limited and high water temperature conditions.
2. There is a significant effect of increasing water temperature toward the photosynthetic activity and biomass of *Symbiodinium* among different coral colonies, either incubate in 48 hours nor 24 hours period.

CHAPTER 2.0

LITERATURE REVIEW

2.1 Corals Morphology and its Taxonomical Work

Corals belong to the kingdom Animalia and phylum Cnidaria (Alex, 2009). The name "Cnidaria" comes from the Greek word, "cnidos" which mean stinging nettle (Grehan, Koslow, & Roberts, 2004). From the name "Cnidaria", we could know that cnidarians are defined by the stinging cells called nematocysts. Phylum Cnidaria is well known by their common names such as sea anemones, corals, jellyfish and hydra (Ruppert et al., 1994). These group of animals is linked together by their similar characteristics.

Cnidarians have been extensively discovered since 1760 until now (Figure 2.1). To date, 27,327 cnidarians species have been discovered but only 6,755 (98%) species of cnidarians had been checked by a Taxonomic Editor (Horton et al., 2018). A study was done by Toda et al. (2007) in Peninsular Malaysia proved that 17.9% to 68.6% of total corals coverage is covered by living corals. A total of 74.5% of total coral coverage communities in Peninsular Malaysia are dominated by Genus *Acropora*, Genus *Porites*, and Genus *Montipora*. While, approximately 2% to 5% are occupied by Genus *Goniastrea*, Genus *Heliopora*, Genus *Galaxea* and Genus *Pavona*.

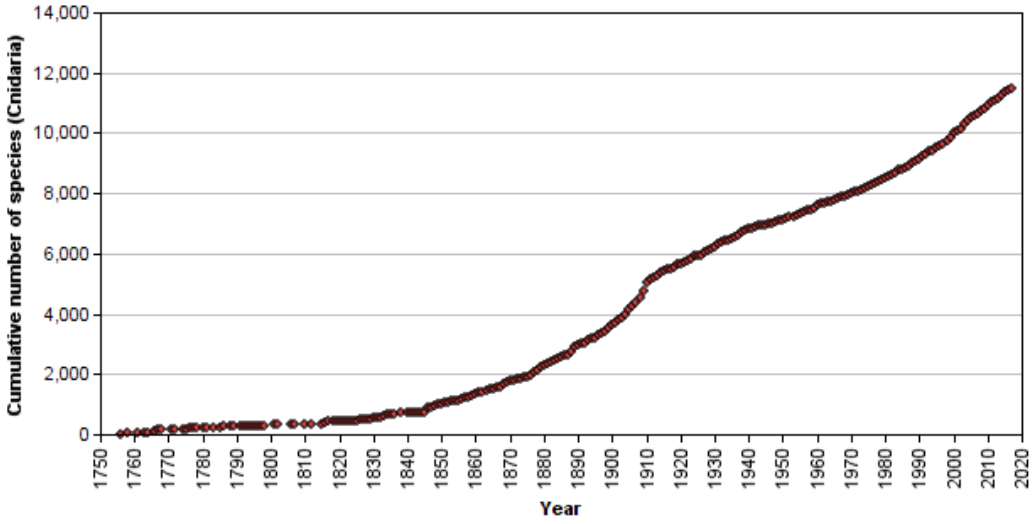


Figure 2.1: The graph showing the discovery rate for Phylum Cnidaria from the year 1760 to 2017 (modified from: Appeltans et al., 2012).

Van (1995) noted that Acroporidae, branching corals, are the most common and diverse family; Faviidae and Poritidae are massive and slow-growing corals; Pocilloporidae is branching with distinct wart-like verrucae; Mussidae have large corallites with large septal teeth; Oculinidae have spikey plocoid corallites and smooth coenosteum; Agariciidae have very small corallites and the septa are continuous between adjacent corallites; Fungiidae are free-living mushroom corals; Pectiniidae are thin plates corals with meandroid valleys; Dendrophylliidae have smoothly rounded corallites and laminar form; Merulinidae are fan-shaped with short straight valleys; Euphylliidae have large polyps and massive colonies; Siderasteridae have petal-shaped corallites; Astrocoeniidae and Trachyphlliidae are rare.

The summary of generic characteristics within Scleractinian was provided to distinguish the coral into its group of families (Veron & Pitchon, 1980; Veron & Wallace, 1984). Until now, 18 scleractinians families had been described by Van (1995) and Veron (2000) (Table 2.1). However, there are multiple orders and families of corals that kept being reshuffled probably due to scleractinians taxonomical

works which still have some changes occurring as they are still being studied through genetic study (Huang et al., 2014).

Table 2.1: Total 18 Families list of scleractinian (modified from: Veron, 2000).

Scleractinian Families		
1. **Acroporidae**	7. Faviidae	13. Poritidae
2. **Pocilloporidae**	8. Mussidae	14. Oculinidae
3. **Agariciidae**	9. Fungiidae	15. Pectiniidae
4. **Dendrophylliidae**	10. Merulinidae	16. Euphyllidae
5. **Siderastreidae**	11. Astrocoeniidae	17. Trachyphlliidae
6. **Rhizangiidae**	12. Meandrinidae	18. Caryophylliidae

2.2 *Symbiodinium* Symbioses and Corals Relationship

Corals are unique animals associated with photosymbiotic unicellular algae called "zooxanthellae". This dinoflagellate symbiont belongs to order Suesiales, classified as genus *Symbiodinium. Symbiodinium,* the yellow-brownish coral symbionts, was first described by Brandt (1881) in the last nineteen centuries (reviewed in Coffroth & Santos, 2005). Corals get their nutrients from symbiotic algae within their cells which found in endoderm tissues layer of polyps (Stambler, 2011; Tim & Jan, 2009). The algae produce oxygen and energy (sugars) that coral polyps need to stay alive (Stambler, 2011). In return, coral polyps produce shelter, carbon dioxide and other nutrients needed for algae growth (Stambler, 2011; Tim & Jan, 2009).

Corals are typically colonies of polyps linked by the gastrovascular system. These polyps are composed of two thin cell layers, which are epidermis and gastrodermis. Zooxanthellae live in endodermal cells of scleractinians' tissues that line the gastrovascular cavity (reviewed in Stat et al., 2006).

Symbiodinium is described as a coccoid yellow-brown endo-symbionts of both dinoflagellate and diatom origin (Banaszak et al., 1993; Blank & Trench, 1986; Trench, 1979, 1993, 1997; Trench & Blank, 1987). Due to the importance of *Symbiodinium* to the survival and growth of reef-building corals, numerous researches have been extensively studied from the last 25 years as climate change is continuously happening and causes a negative effect to coral reefs. These researches have been reviewed and summarized in Table 2.2.

Table 2.2: Summary list of reviews on *Symbiodinium* biology and symbiosis in the past 15 years (Kirk & Weis, 2016).

Topic	References
Diversity and systematics	(Stat et al., 2012)
Flexibility and specificity of the partnership	(Baker, 2003; Coffroth & Santos, 2005b; Fay & Weber, 2012; Goulet, 2006; Pochon & Pawlowski, 2006; Stat et al., 2006)
Cell biology and physiology of *Symbiodinium* symbioses	(Davy et al., 2012; Fransolet et al., 2012; Gordon & Leggat, 2010; Hill & Hill, 2012; Lesser et al., 2013; Muller-Parker & Davy, 2001; Stambler, 2011; Venn et al., 2008; Weis et al., 2008; Yellowlees et al., 2008)
Genomics	(Jeong et al., 2014; Leggat et al.,2011; Meyer & Weis, 2012)
Mechanisms of coral bleaching	(Baird et al., 2009; Lesser, 2006a, 2011; Weis, 2008)
Dinoflagellate genomic organization	(Wiscaver & Hackett, 2011)

In earlier research, *Symbiodinium* was described by Freudenthal (1962) as a single species, *Symbiodinium microadriaticum*, found in cnidarian species. This had made researchers hypothesized that corals may survive under stressful condition by exchanging its zooxanthellae among coral species. However, Goulet (2006) reported that the coral's ability in changing its symbionts seem to linked to the ability of whether a coral species can host multiple zooxanthella clades, either at different depths on the same reef, or different reefs or at different geographic locations or concurrently within

the same colony. With the advance technologies nowadays, the *Symbiodinium* is now recognized to have diverse genus where there are nine monophyletic clades (clade A-I) based on its small subunit ribosomal sequences variation (Pochon & Gates, 2010).

Nevertheless, *Symbiodinium* clades A, B, C, and D are mostly found within scleractinians, clade F and G are more anecdotic, while Clade H and I are found extensively within foraminifera (Pochon & Gates, 2010). *Symbiodinium* clade B and C are relatively flexible to its environmental condition, especially clade C, where they are the most beneficial for their host's fitness, releasing the highest amount of photosynthesis derived carbon. Plus, *Symbiodinium* clade C is reportedly dominant in Indo-Pacific corals while clade B is the most common species found in Atlantic-Caribbean region (reviewed in LaJeunesse, 2005).

Symbiodinium clade C also presumably has a wide temperature and salinity tolerance which make it dominant in the tropical area (Baker, 2003). Baker (2003) also reported that *Symbiodinium* clade D is adapted to stress tolerance while Rodriguez-Lanetty et al. (2001) mentioned that *Symbiodinium* clade B is adapted to lower light and cooler seas of higher-latitude environments in temperate areas.

Symbiodinium diversity is also found to be different in morphology, biochemistry, physiology, and ecology at the clade and sub-clade level (reviewed in Kirk & Weis, 2016). *Symbiodinium* size ranges from 6µm to 15µm diameter and varies between genotype and host (Frade et al., 2008; LaJeunesse, 2001; Lajeunesse et al., 2005). In hospite (living inside host cell) and in culture, *Symbiodinium* has coccoid shape limited by a continuous cellulosic cell wall. There is a single chloroplast with thylakoids arranged in stacked and around the periphery of

the cell. A pyrenoid body is connected to the chloroplast which functions as storage of photosynthetic products. *Symbiodinium* genomes range in size from 1.5 to 4.8 pg/cell (LaJeunesse et al., 2005) and contain different numbers of chromosomes (Blank & Trench, 1985).

According to Blank & Trench (1985), Jeong et al. (2014) and Trench & Blank (1987), although cultured *Symbiodinium* appear similar hospite *Symbiodinium*, there are differences in the ultrastructure and cellular morphology observed (Figure 2.2). In hospite, *Symbiodinium* are found only in coccoid shape and they divide mitotically. In another hand, cultured *Symbiodinium* is found in coccoid cells with a flagellum, which cause the cells to become motile either following karyokinesis and cytokinesis or even without mitosis (Trench, 1993). The cells become motile swarmer which has gymnodinoid morphology.

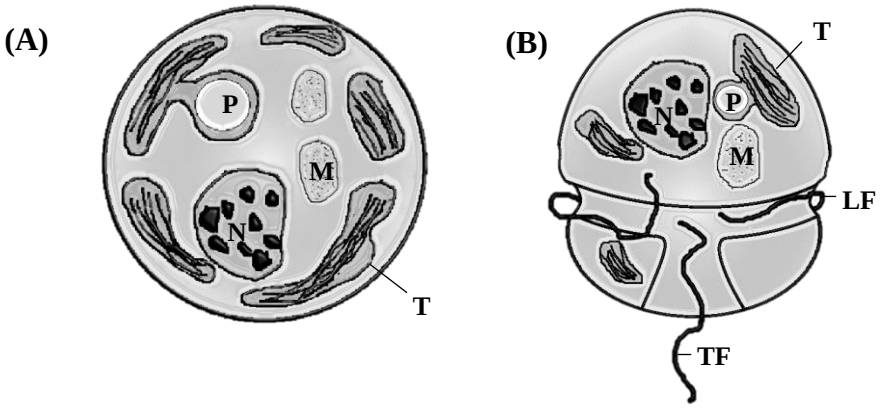


Figure 2.2: *Symbiodinium spp*., zooxanthellae cell anatomy. (A) *Symbiodinium* in hospite; (B) Cultured *Symbiodinium*. P: pyrenoid; M: mitochondria; N: nucleus with condensed chromosomes; T: thylakoid; LF: longitudinal flagella; TF: transverse flagella (modified from: Stat et al., 2006).

To date, the most challenging problem faced by researchers in aiming to understand the diversity of *Symbiodinium* is their classification groups to the lowest taxonomy level. According to Kirk & Weis (2016), almost 20 species of *Symbiodinium*

had been identified through molecular work. However, half of the species are still lack of proper species descriptions due to few morphological differences in hospite and inability to grow various species of *Symbiodinium* in culture (Kirk & Weis, 2016; Stat et al., 2012).

These zooxanthellae are able to photosynthesize and contain chlorophyll *a* and c, and characteristic dinoflagellate pigments such as diadinoxanthin, diatoxanthin, β-carotene, and peridinin (Muller-Parker & D'Elia, 2015; Stambler, 2011). The chlorophyll is part of photosynthesis apparatus while diadinoxanthin and diatoxanthin are parts of photoprotective xanthophyll (Brown et al., 2002).

Majority of coral-*Symbiodinium* associations display horizontal transmission while a significant minority display vertical transmission (Kirk & Weis, 2016). Horizontal transmission occurs when the host partner acquires new symbionts by engulfing from the environment with each host generation (Kirk & Weis, 2016; Stambler, 2011). Vertical transmission occurs when the symbionts are transmitted directly from host parents to offspring, usually happens during oocytes stage (Kirk & Weis, 2016; Stambler, 2011).

In scleractinian, Baird et al. (2009) reported 85% of symbiotic corals to display horizontal transmission compared to 15% which transmit symbionts by vertical transmission. These different transmission modes had marked different evolutionary strategies (Douglas, 2010). Douglas (1994) mentioned that horizontal transmission enables the corals in the potential of shuffling or switching its partnerships in adapting to changing the environmental conditions. However, vertical transmission could result in decreasing of corals ability to respond to the environmental changes and increased of genetic bottlenecking (Kirk & Weis, 2016).

A study done by Goulet (2006) concluded that 77% of coral species contains single species of zooxanthellae while only 23% are dominated by corals species which host multiple zooxanthellae clade. These different symbiotic associations are typically affected by geographic location and physical conditions such as light and temperature (Lajeunesse, 2002; Lajeunesse et al., 2004; LaJeunesse et al., 2004). In conclusion, the local environments influence, control, and determine the specificity of the host and the *Symbiodinium* genotypes (reviewed in Stambler, 2011).

Coral which is the host of variety *Symbiodinium* species often show distribution patterns according to light exposed across an individual colony (Stambler, 2011). This has been proven by VanOppen et al. (2001) where the study showed that *Symbiodinium clade* C2 dominated in part exposed to the sun, and *Symbiodinium* C1 dominated in the shaded part of *Acropora tenuis.* Another study was done by Rowan et al., (1997) showed that *Symbiodinium* clade A are predominate at the top of *Montastraea* sp., while *Symbiodinium* clade C dominate in the sides of the same colonies.

Zooxanthellae rarely dominate at the tip of scleractinians due to high light exposure (Fang et al., 1989). Zooxanthellae that are exposed to high light will have lower chlorophyll per coral unit area compared with those zooxanthellae settle on a shaded section of the colony (Dubinsky et al., 1984; Fujise et al., 2013; Vaughan & Wells, 1943). Therefore, fewer zooxanthellae are found at the tip of scleractinians. Nevertheless, there are some cases where the branches exposed to low light will have more zooxanthellae than those parts exposed to light and no zooxanthellae found on coral parts facing the dark (Dubinsky & Jokiel, 1994; Fitt & Cook, 2001; Titlyanov et al., 2001).

2.3 Threats to Coral Reef Ecosystems

Coral reef ecosystem is the most diverse and productive ecosystems which support the local fisheries industry and tourism industry. In addition, the coral reef ecosystem is also known as an indicator of a global warming event (Graham et al., 2006). When the partnership of coral and its symbiont breaks down during stressful condition, a process known as coral bleaching will occur.

All organisms which live in reef area play important roles in the resilience of coral reefs (Bellwood et al., 2004; Connell et al., 1997; Mumby et al., 2007). Resilience also functions as diversity responses and as the factor of abundance of the remaining species in coral reef habitat (Chapin et al., 2000) However, the study was done by Graham et al., (2006) shows that climate change is one of the main reason in loss of live corals, substantial reductions in species richness, reduced taxonomic distinctness, and loss of species among coral reefs organisms especially reef fish.

In the year 1998, El-Nino event correlated with the Indian Ocean dipole had caused global mass bleaching event occurred in the western Indian Ocean (Saji et al., 1999). This phenomenon resulted in a 75% to 99% loss of live coral (Goreau et al., 2000). In addition, more than 90% of live coral number declined across the entire ranges of the Seychelles islands (Sheppard, 2003). In Malaysia and Indonesia, coral reefs are declining. This is due to local activities and natural disasters such as the Tsunami in 2004. This tsunami event had heavily damaged the coral reef area.

Pulau Sipadan, Sabah, Malaysia (East Malaysia) is found to be the best environment condition for coral reefs. Meanwhile, the coast off Miri in the state of Sarawak was found to have the highest abundance of corals (Ridzwan & Cabanban, 1994). Tun et al. (2008) stated that east coast islands in

Peninsular Malaysia are richer in reef biodiversity than west coast offshore islands. The numbers of coral reef are declining on the northern offshore islands consist of Pulau Langkawi, Pulau Payar and Pulau Perak in the state of Kedah and Pulau Pangkor, Pulau Jarak and Pulau Sembilan in the state of Perak. In addition, corals reefs are not well developed in Port Dickson and Tanjung Tuan in the state of Negeri Sembilan. According to Yasin et al., (1998), increasing of sedimentation and tourism impacts are the major threat of coral communities in Peninsular Malaysia. This is also supported by studies by Lee & Mohamed (2011) and Safuan, et al., (2016) which stated that there are higher sedimentation rates in west coast compared to the east coast of Peninsular Malaysia.

Global climate change has also caused a huge coral bleaching in the Coral Triangle region including Malaysia, Indonesia and Thailand (Tan & Heron, 2011; Tun et al., 2008). In Sabah, storms and active dynamite fishing activities cause the coral reefs damaged (Yasin et al., 1998). While in Sarawak, coral reefs are affected by high sedimentation and sand mining (Idris et al., 2006; Pilcher & Cabanban, 2000). The study conducted by Burke et al., (2002) summarized that 87% of corals were covered on the percentage at medium or higher threats of coral reefs in Malaysia.

Most of the coral reef organisms rely on corals for turning key life processes, such as recruitment, shelter or diet (Graham et al., 2006). With the rapid increase of thermal climate, live coral area reduces consequently due to a loss in the physical structure of coral reef ecosystem, resulting in species richness to decline on coral reefs after disturbance (Risk, 1972; Sano et al., 1987; Syms & Jones, 2000). Loss of this coral reef habitat had increased the competition over the remaining space and thus increases resistance to predation (Munday & Jones, 1998). This also may impact the efficiency of fishing activities (Graham et al., 2006).

2.4 Influences of Excess Temperature and Light on the Coral Reef Ecosystem

Light is the key factor affecting the coral growth rate. According to Frade et al. (2008), Kühl et al. (1995), and Lesser et al. (2010), light variation will affect the zooxanthellae density, photosynthetic pigment concentration, and photosynthetic efficiency. As the result, the changes of zooxanthellae density will change the coral physiology and its response to coral stress behavior (Venn et al., 2008). Excess light could cause photo-damage to photosynthetic systems, mainly the PSII (Baird et al., 2009). This photo-damaged protein in PSII is repaired by replacing newly synthesized proteins (Aro et al., 2005). Nishiyama et al. (2006) noted that PSII photo-damage happens in two steps, where the primary damage is caused by ultraviolet radiation (UV) and strong blue light which happens in oxygen-evolving complex of PSII, while secondary damage is caused by light absorbed by photosynthetic pigments in PSII. However, Frade et al. (2008) and Hoegh-Guldberg & Jones (1999) stated that there is an increase of zooxanthellae density when the corals are exposed to high light intensities. High or low light intensities lead to coral halobiont adaptive response by (i) increasing or decreasing the photosynthetic pigment concentration in zooxanthellae; and (ii) increasing or decreasing of zooxanthellae density (Titlyanov et al., 2001). Zooxanthellae density is also influenced by the PAR value to response on light intensity, where low PAR value causes an increase in zooxanthellae density (Titlyanov et al., 2001), while high PAR value shows a decrease of zooxanthellae density (Titlyanov & Titlyanova, 2002).

Different with photo-damaged, photo-inhibition is a process where an accumulation of oxidative stress occurs during photosystem II (PSII) which causes thermal bleaching to happen (Iglesias-Prieto *et.al.*, 1992). Different photo-inhibition sensitivity toward increased temperature among *Symbiodinium* causes a difference in

sensitivity among coral species (Fitt & Warner, 1995; Warner et al., 1996). However, the response of host corals is not always related to thermal tolerance of symbiont (Baird et al., 2009). For examples, coral from genus *Stylophora, Pocillopora* and *Acropora* are highly vulnerable to bleaching, while genus *Cyphastrea*, *Goniopora, Galaxea,* and *Pavona* are highly unaffected (McClanahan et al., 2004). In addition, physiological changes in host corals, such as reducing in epidermis and apoptosis of gastrodermal cells thickness, might affect the symbionts when exposed to heat (Ainsworth et al., 2008).

Coral bleaching event acts as a communication breakdown between coral and zooxanthellae under stress (Baird et al., 2009). At most locations, coral bleaching occurs in a range of 29-32°C which is low temperature for tropical organisms to suffer high mortality (Baird et al., 2009). During coral bleaching events, macro-algae are not affected (Berry & Bjorkman, 1980). Generally, water temperature above 35°C will change the photosynthesis in free-living micro-algae (Berry & Bjorkman, 1980).

Photo-inhibition also could be prevented by antioxidant systems between the host and symbiont zooxanthellae (Baird et al., 2009). In response to oxidative stress, antioxidant systems will remove potentially toxic reactive oxygen species caused by stress such as superoxide dismutase (SOD) and catalase, ascorbic acid, carotenoids (Lesser, 2006b) and mycosporine glycine (Yakovleva et al., 2004).

Corals also cope with high temperature and high light stress by widely spread heat-shock proteins (Baird et al., 2009). Heat-shock proteins maintain the protein structure and cell function in coral tissue (Baird et al., 2009). The activity done by heat-shock proteins could influence the bleaching response (Baird et al., 2009). This has been proven by Brown et al. (2002) where the coral *Goniastrea aspera* have higher

concentrations of heat-shock proteins and these tissues do not bleach when exposed to the high light condition.

Corals also change its diet in response to bleaching stress (Baird et al., 2009). The coral host reduces its symbionts zooxanthellae density by increasing its heterotrophic feeding (Grottoli et al., 2006). This will allow the symbiont zooxanthellae to assign more energy to its own antioxidant defenses and followed in preventing damage to the algal cell (Grottoli et al., 2006).

2.5 Pulse-Amplitude Modulation (PAM) in Assess Photosynthetic Activity of Coral Symbionts

Photosynthesis is the process of light-driven reactions. The energy from sunlight is converted and fixed the carbon from carbon dioxide into organic carbohydrates. In aquatic environments, this photosynthesis process is the major activity done by marine primary producers such as micro-algae organisms. Photosynthesis process in corals occurs where the antenna pigments of photosynthetic apparatus in zooxanthellae's chloroplasts absorb the light (Rodrigues et al., 2008) and transfer the excitation energy to photosystem II (PSII) and down the photosynthetic electron transport chain (Krause & Weis, 1991). The detail photosynthetic process in zooxanthellae cells as illustrated in Figure 2.3.

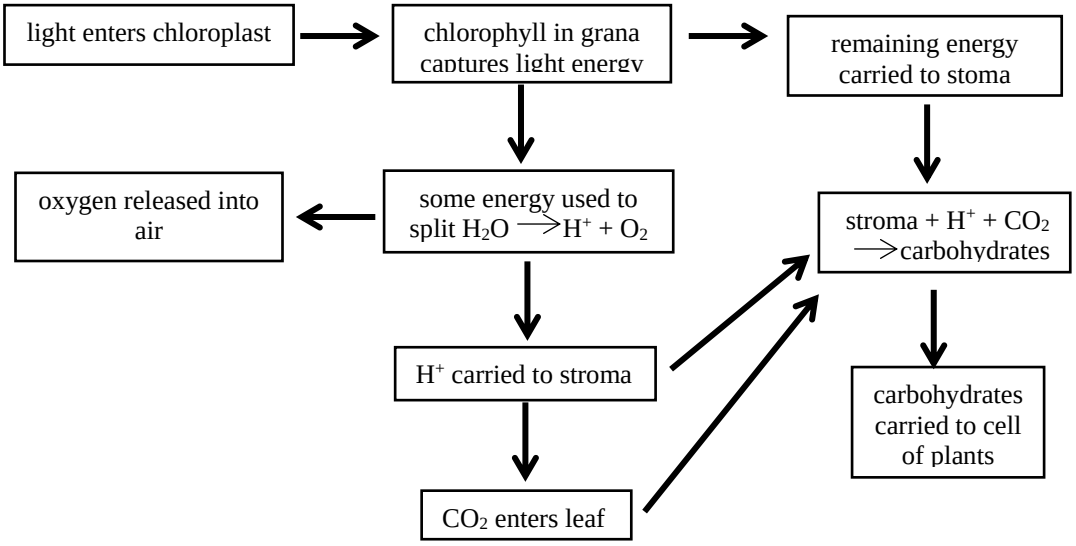


Figure 2.3: Summary of the photosynthesis process (Roth, 2014).

Pulse Amplitude Modulated (PAM) fluorometers can be used to study the photosynthetic activity of organisms. This PAM system is sensitive enough to detect the fluorescence yield of PSII. Furthermore, PAM fluorometry can separate the fluorescence signal from actinic light (Ogren & Baker, 1985). PAM fluorometer emits two different colours of light, blue and red. The blue light is to be optimal for green algae and diatoms, whereas the red light is optimal for cyanobacteria (Honeywill et al., 2002; Yentsch & Yentsch, 1979). Fluorescence techniques include the measurement of light energy emitted from the light-harvesting pigments associated with the process of photosynthesis. This technique is a highly useful tool in studying the photo-physiological and ecological work.

To date, Pulse Amplitude Modulated (PAM) fluorometers have become a key technique for investigating the changes in the photosynthetic physiology of dinoflagellate symbionts of reef-building corals, *Symbiodinium* spp. (Ralph et al., 1999). Fluorescence is used as a rapid, non-destructive proxy of photochemical efficiency and photosynthetic rate measurement. Fluorescence is the re-emission of a photon of light, with a lower energy than the photon absorbed. At

room temperature, most of the fluorescence measure came from the light-harvesting complexes of photosystem II (PSII) (Venn et al., 2008).

Ralph (2005) explained that the quantum yield of photosystem II (PSII) is linked to photosynthetic activity and under some conditions is roughly proportional to oxygen production or uptake. However, this relationship rarely holds up at elevated irradiances due to a range of competing processes including photorespiration (Ralph, 2005).

The biophysical condition of the photosystem II (PSII) is related with maximum (F_m') and minimum (F_0) fluorescence. When there is no photon absorbed by antennae complexes (in dark adaption), the reaction centers are described as "open". This means that there is no electron transferred from PSII. Thus, the reaction center is open when the plastoquinone molecule (Q_A) is oxidized. Hence, the yield of fluorescence is at its minimum level (F_0). If there is photon absorbed by antennae complexes (in light adaption), the pigment molecules will be excited and cause the reaction centers of PSII to close to reduce all Q_A. Hence, the maximum yield of fluorescence (F_m') occurs. The following formulae can be used to assess the relative condition of the photosynthetic apparatus.

$$\text{Maximum Quantum Yield } (F_v/F_m) = (F_m-F_0)/F_m$$

$$\text{Effective Quantum Yield } (F_q'/F_m') = (F_m'/F)/F_m'$$

Maximum quantum yield (F_v/F_m) is the maximum light been efficiently utilized during the dark period. Therefore, this parameter can be used in showing the physiological state of zooxanthellae. F_v/F_m could be used to assess the nutrient status and health of some microalgal populations. Nevertheless, the prevailing light conditions may affect the measured efficiency if the sample is not long enough being dark adapted to oxidized the Q_A and reverse non-photochemical quenching (NPQ)

process (Kalaji et al., 2014). According to Ralph et al. (1999), coral samples require 20 to 30 minutes for dark adaptation in measured its F_v/F_m value. This timing is enough for corals in responding to no photon transmitted in reaction center condition and allows regulatory process such as photoinhibition to recover.

A study by Dubinsky et al. (1995) found that the algal growth rate, maximum photosynthesis, respiration, *in-vivo* absorption, and β-carotene will increase as the light increases. Consequently, photosynthetic units size, the concentration of chlorophyll *a* and *c,* peridinin concentration, thylakoid area and quantum yield are decreased (Falkowski & Dubinsky, 1981; Stambler & Dubinsky, 2004). These responses of zooxanthella's photosynthetic pigments towards light intensity is illustrated in Figure 2.4.

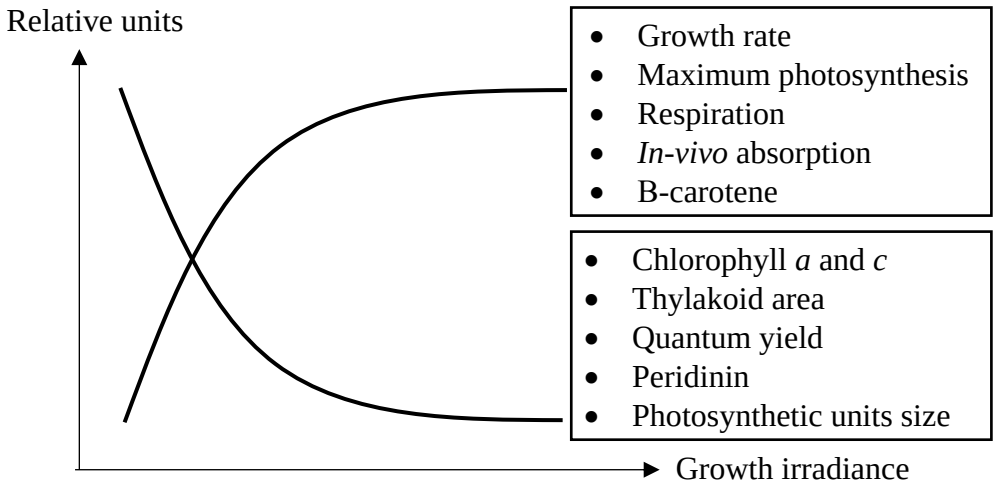


Figure 2.4: Changes in photosynthetic pigments of zooxanthellae in response to light intensity during growth (modified from: Dubinsky et al., 1995).

According to Warner (2005), corals exposed to thermal stress have shown a significant loss in F_v/F_m compared to corals in a non-stress condition which typically precede detection of any loss in zooxanthellae density. This is also supported by Rodrigues et al. (2008) and Warner et al. (1999) where their studies proved that declining of F_v/F_m is correlated with the loss of PSII in zooxanthellae.

The relationship between light intensity and photosynthetic activities within coral polyps has also been proven by Ralph et al.,(1999), where they assessed the light saturation behavior of three different coral species in their rapidly changing natural environment. According to Ralph et al. (1999), *Goniastrea* sp., *Porites* sp. and *Acropora aspera* display quite a similar trend, where the differences are most apparent in the F_v/F_m value at low light intensity and low electron transport rate (ETR) values at a high light intensity (Figure 2.5).

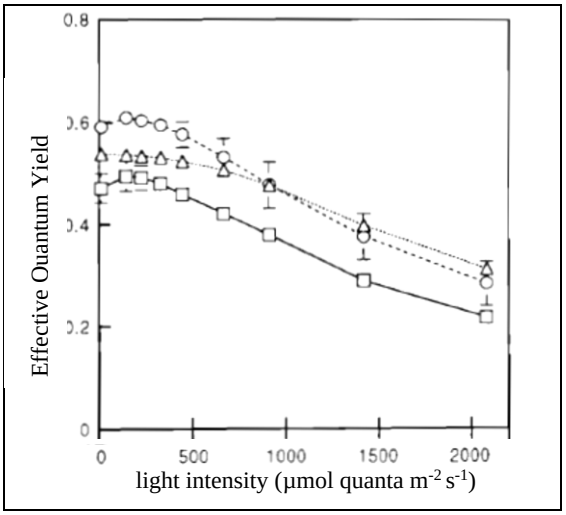


Figure 2.5: *In-situ* effective quantum yield (F_v/F_m of *Goniastrea* sp., *Porites* sp. and *Acropora aspera* (Modified from: Ralph et al., 1999).

Figure 2.5 shows the curves of *Goniastrea* sp., *Porites* sp. and *Acropora aspera* follow the changes of photosynthesis towards changes in light intensity. Maximum quantum yield (F_v/F_m) is higher in the beginning and gradually drops towards the increase of light intensity. The high photosynthetic capacity can be considered to be the result of effective adaptation to the high-light conditions on the reef (Ralph et al., 1999).

Limited study was conducted on coral from Malaysian waters. The only study case was done in Pulau Tioman, Malaysia regarding the effective and maximum quantum yield on corals. This study was done by Adzis et al. (2009) mainly to provide

baseline data on the health of *Pocillopora damicornis* in its habitat. Adzis et al. (2009) found that there was a significant diurnal variation of *P. damicornis* which gave a depression of F_v/F_m values when the highest PAR intensity recorded at noontime.

As the thermal sensitivity of zooxanthellae seems to be the primary cause of coral bleaching, researchers have intensively used several methods of measuring active chlorophyll fluorescence to study the photobiology of these symbiotic dinoflagellates for a better understanding on how photosynthetic processes are affected by excessive thermal and light exposure on the coral's tissues. This PAM fluorometer has been introduced a decade ago, and now it has become a tool for investigating coral bleaching.

CHAPTER 3.0

MATERIALS AND METHOD

This study was focused on a non-experimental and experimental study which is divided into four parts (Figure 3.1) They are (i) identification of corals; (ii) the coral colonies and its symbionts relationship with environmental abiotic factors; (iii) the survival rate of isolated coral symbionts in different media; and (iv) effect of thermal stress on the health of coral symbionts. All the methodologies used in each part were summarized in Figure 3.1 and Figure 3.2.

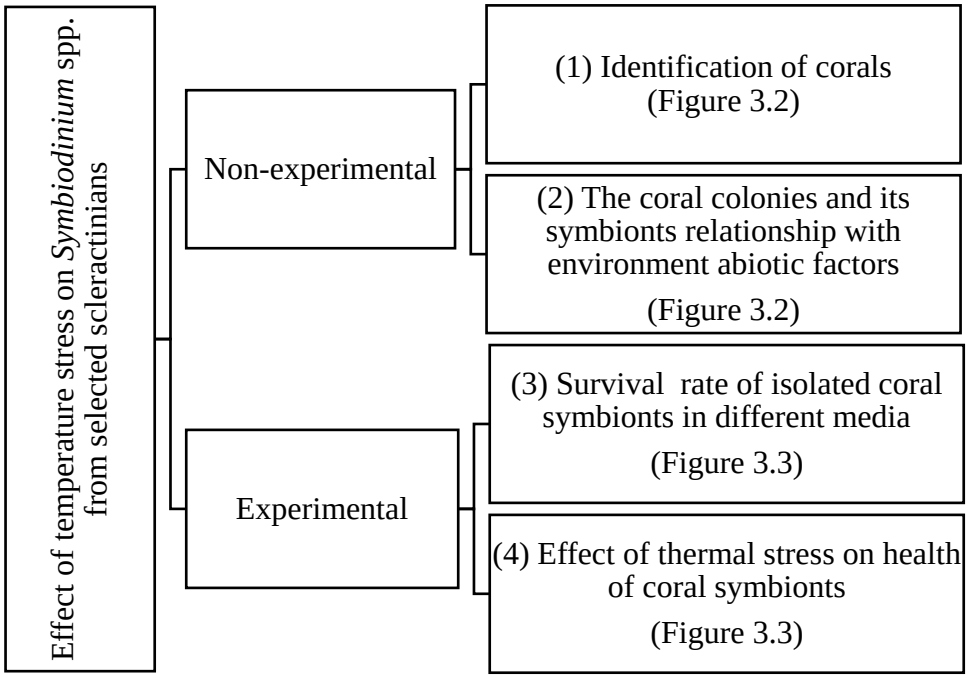
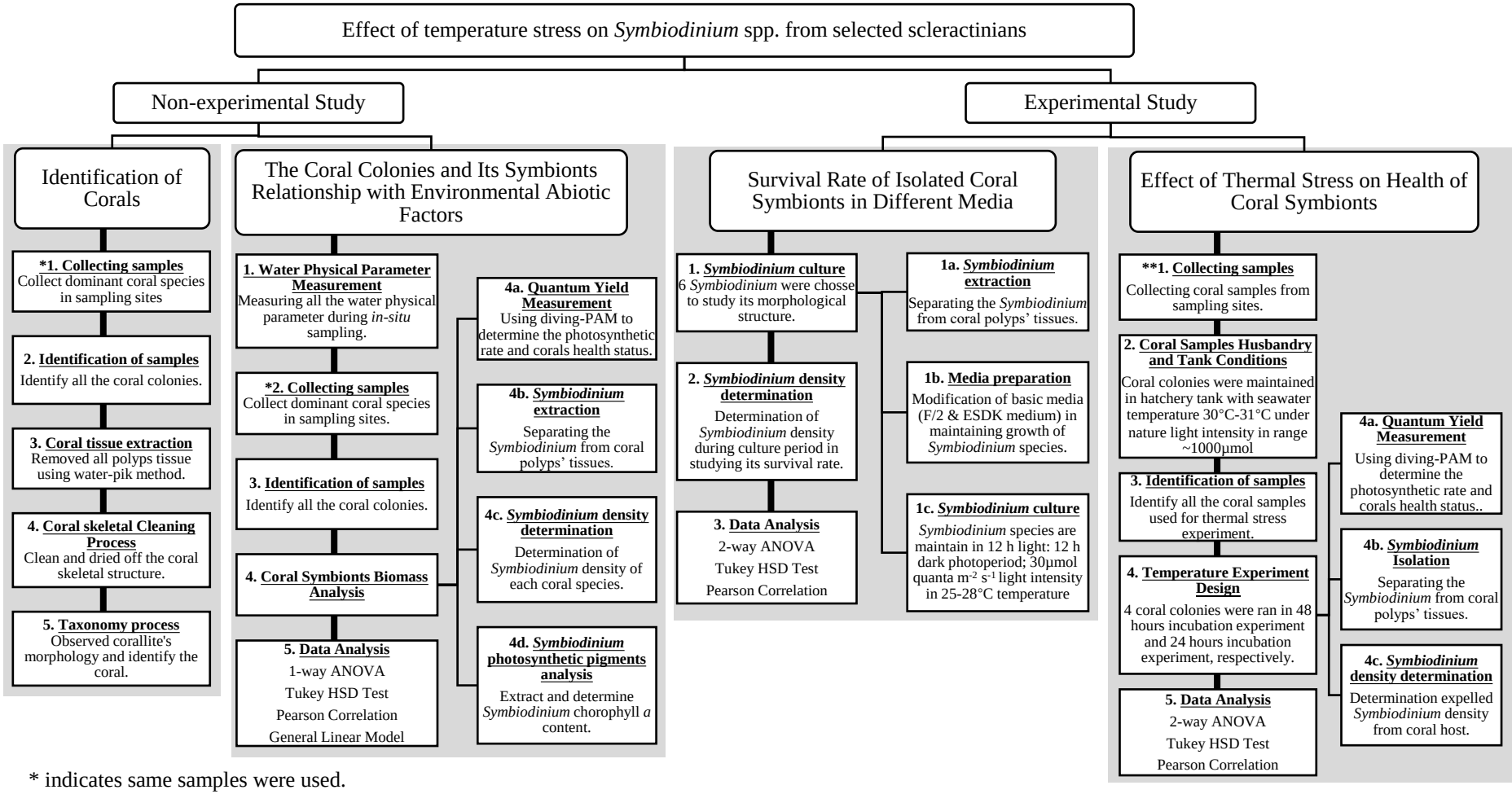


Figure 3.1: The framework of this study.



* indicates same samples were used.

** indicates different samples were used (*Cyphastrea chalcidicum, Pavona danai, Goniopora cellulosa, Turbinaria mesenterina, Goniastrea retiformis* and *Porites lutea.)*

Figure 3.2: Summary methodologies for this study.

3.1 Study Sites Description

Samples of corals containing *Symbiodinium* spp. were collected six times between November 2015 to September 2017 from three sampling sites in Peninsular Malaysia. These three sampling sites were Pulau Songsong, Kedah, Pulau Redang, Terengganu and Pulau Kendi, Pulau Pinang (Figure 3.3).

Pulau Songsong (5°48'39.7"N 100°17'39.0"E) is located in between Pulau Pinang and Pulau Payar (Figure 3.3). While, Pulau Kendi (5.2329° N, 100.1794° E) is located near Southwest Pulau Pinang (Figure 3.3). Both sampling sites are situated in the northern part of Peninsular Malaysia where the waters are turbid.

Contrariwise, Pulau Redang (5°46′30″N 103°0′54″E) which is on the east coast of Peninsular Malaysia, is a very famous island among tourists in Terengganu due to its water clarity. The water is very clear as the corals and fishes can be seen just a few meters from the beach. In addition, Pulau Redang is rich with various types of coral species which supports most of the marine life.

3.2 Non-experimental Study

3.2.1 Water Physical Parameters Measurement

All water physicals parameters were measured and recorded during the sampling day. Underwater light intensity was measured by using LI-COR Spherical Underwater Quantum Sensor (USA) while dissolved oxygen, pH, water temperature was measured by using YSI Pro-1020 (USA) Dissolved Oxygen meter. Seawater salinity was measured using a refractometer and multi-parameter prob. Each parameter was collected for three replicates (n=3) per sampling. Therefore, total 18 replicates (n=18) were collected for each parameter in this study.