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The role of thermal history in shaping the microbiome of Red Sea corals

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To my parents and my wife, I dedicate this work

Abstract

Coral reefs are immensely vulnerable to climate change and particularly the effects of ocean warming; in efforts to understand whether and how reef systems will survive into the future, research is increasingly focusing on present day populations acclimatized to thrive under relatively extreme conditions. Whilst corals thrive along a range of environmental conditions, including relative extremes, within the Red Sea, these coral populations are still considered not well explored of the genetic and physiological signatures throughout this system. Corals microbiota communities (the "microbiome") are recognized as a major component as to how corals "acclimatize" to different environmental conditions; therefore, this work aimed to investigate the historical thermal variability along the Red Sea and subsequently identify the relative role of coral microbiome associated with differences in coral thermal tolerance. Remotely sensed data (1982-2012) demonstrated migration of Sea Surface Temperature anomalies (i.e. DHW) from the south to the north during this time frame. Analysis of historical bleaching records indicated that coral populations were more tolerant to bleaching in the northern compared to the central/southern Red Sea. *Symbiodinium* clade type (ITS2) and microbial community (16S rRNA metagenomics) associated with six key coral species persisting across five sites of the northern Red Sea (29°-20°N) were then examined. *Symbiodinium* clade identity associated with each coral species generally remained highly conserved throughout the sites sampled. In contrast, microbial communities were variable within and between species across the Red Sea sites. Corals from two sites (central-Jeddah and northern-Hurghada) were exposed to a thermal stress experiment which confirmed that corals were

more heat resistant at Hurghada (summer SST mean is 3.3 °C less) than Jeddah; however, symbiont ITS2 clade types were the same at both sites. Conversely, microbial community changed in heat stressed samples at Jeddah compared to the control group, while it remained stable at Hurghada. This work provides for the first time genetic analysis on corals' microbiome inhabiting extreme thermal resistant region (i.e. the northern Red Sea) that contradict the global bleaching pattern. Our findings suggest that plasticity of microbial community may play the key role in acclimation of corals experience thermal anomalies in the Red Sea suggesting presence certain microbial phylotypes fill specific thermal niche. Finally, the higher latitudes of the Red Sea will broadly serve as a potential corals refugia which highlights the importance to conserve and implement a regional management policy to improve corals thermal tolerance of this region to be used as a genetic reservoir.

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Your sincerely,

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Chapter 1

1. *Review of literatures*

1.1. General Introduction

Coral reef ecosystems are one of the largest reservoirs for global biodiversity harboring around one-third of all described marine organisms (Reaka-Kudla, 2001). Undoubtedly, coral reef ecosystems are amongst the most threatened global ecosystems (Costanza *et al.*, 1997; Wilkinson, 2000) and the current "coral reef crisis" is almost certainly the result of complex and synergistic interactions among local scale human imposed (direct) stresses and global scale (indirect) climatic stresses (Veron *et al.*, 2009).

Such stresses all lead to a loss of coral cover, productivity, and/or biodiversity and thus the "health' of coral reefs; notably coastal development, outbreaks of coral grazers (e.g. *Acanthaster planci*, crown of thorns starfish), recreational SCUBA diving, anchor damage, sedimentation from urban development and deforestation, overfishing, destructive fishing practices, eutrophication from agriculture and sewage discharge, pollution from herbicides and pesticides, invasive species and epidemic diseases (Hoegh-Guldberg *et al.*, 2007; Veron, 2008). As well as factors associated with "global warming"; notably, elevated sea surface temperatures (Hoegh-Guldberg *et al.*, 2007) and increased frequency/intensity of El niño-southern oscillation (ENSO) thermal anomalies (Baker *et al.*, 2008) and storms (Lugo-Fernandez & Gravois, 2010), ocean acidification (McCulloch *et al.*, 2012), rising sea levels (Richardson *et al.*, 2009), and alterations in current circulation (Leichter *et al.*, 2012). Several of these factors can act synergistically to accelerate the rate of coral reef health degradation (Wilkinson, 2000). Most recent estimates suggest that 19% of the world's coral reefs have already been lost and a further 35% are seriously threatened (Wilkinson, 2004) with one-third of all reef-building corals considered to be at risk of extinction (Carpenter *et al.*, 2008).

Of all climatic stressors, elevated sea surface temperatures (SSTs) via ENSO events have been, and continue to be, of greatest concern. Such events have periodically caused widespread, highly conspicuous, and hence of high public profile, (Suggett & Smith, 2011) "coral bleaching" and mortality since the 1980s (Veron *et al.*, 2009). SSTs have increased by 0.8 °C since the late 19th century (Donner *et al.*, 2005) but are predicted to rise by a further 2–3 ^{o}C during the 21st century (IPCC, 2007). In turn, large scale bleaching and mortality events could occur annually on the world's coral reefs by 2050 (Nicholls *et al.*, 2007). Hoegh-Guldberg (1999) even predicted that mass bleaching could become an annual occurrence by 2020 in Southeast Asia and the Caribbean, by 2030 on the Great Barrier Reef and by 2040 in the central Pacific. As such, in order to best manage the future of coral reefs, international efforts have intensified to identify the nature and extent with which coral species and reef systems are susceptible to thermal stress and importantly identify natural "pockets of resilience".

Naturally, high-temperature environments have already been identified to retain healthy, growing coral populations (Coles, 1997; Kleypas *et al.*, 1999), with elevated bleaching tolerances (Jokiel & Coles, 1990; Oliver & Palumbi, 2011a). These thermally tolerant corals are among the most likely to cope with future climate change (West & Salm, 2003), and thus, represent an essential source of information about mechanisms underlying observed differences in coral physiological resistance (Palumbi *et al.*, 2008).

The coral reefs of the Red Sea are amongst the best-developed reefs in the western Indian Ocean (PERSGA, 2006) in spite of extreme environmental conditions such as high salinity, turbidity, and temperature. Coral reefs in such extremes are considered 'marginal' since they thrive at the limits for reef growth (Kleypas *et al.*, 1999). Importantly, Red Sea corals species do not appear to "bleach" at the average SSTs typically associated with mass

bleaching for other reefs worldwide (West & Salm, 2003); consequently, Red Sea reefs, especially those of the north have so far 'escaped' mass bleaching (Rouphael & Abdulla, 2007) and thus raises some fundamental questions: (1) what is the thermal range for bleaching tolerance of Red Sea coral species? (and are these temperature thresholds within future warming scenarios)? (2) What is the nature and extent with which Red Sea corals bleach? (and do all coral species bleach at the same rate and via the same mechanism?). **The aim of this literature review is therefore to discuss the processes and mechanisms by which elevated sea surface temperature can impact reef-building corals (and their associated communities), with reference to the known environmental conditions and coral reef assemblages of the Egyptian Red Sea.**

1.2. The coral holobiont

Coral reefs are built by scleractinian corals that deposit external calcium carbonate skeletons. Each coral is composed of a colony of polyps (Phylum: Cnidaria, Class: Anthozoa) whose tissue contains single-celled dinoflagellate microalgae, or "zooxanthellae", of the genus *Symbiodinium*. Corals and their zooxanthellae have a mutualistic symbiosis, as both partners appear to benefit from the association. The zooxanthellae are located within vacuoles, termed the Symbiosome, in the cells of the host endoderm (Trench, 1987), and photosynthesize to produce sugars, amino acids, carbohydrates and small peptides; these 'simple' compounds are selectively leaked across the symbiosome barrier to ultimately supply their coral host with up to 95% of its nutrition (Trench, 1979; Muscatine, 1990). In return, the zooxanthellae receive essential nutrients to drive photosynthesis, namely nitrogen (ammonia), phosphate and CO_2 as 'waste' from the host's metabolism (Trench, 1979). This tight recycling of nutrients within the association minimizes the loss of nutrients (Muscatine

& Porter, 1977), and facilitates the high productivity of corals in otherwise nutrient-poor oligotrophic waters (Hoegh-Guldberg, 1999a).

Zooxanthellae are not the only microbes that comprise a 'healthy' coral community. Corals form close associations with a complex consortium of microorganisms, including, fungi, endolithic algae, bacteria, archaea, and viruses, with the collective group of organisms, which together with the zooxanthellae, are referred to as the coral holobiont (Rosenberg *et al.*, 2007). Microbial communities play various roles in immunity, fueling the micro-planktonic food web, nutrient cycling, antimicrobial protection for the coral holobiont and maintaining coral health and resilience (Rohwer & Kelley, 2004; Reshef *et al.*, 2006). Understanding microbial communities associated with corals, their functional roles and how they change through time is the key to understanding how these changes will affect the coral holobiont since shifts in the bacterial community composition may affect the coral health and in turn the susceptibility of the host to bleaching (Rosenberg & Ben-Haim, 2002; Ritchie, 2006).

1.3. What is coral bleaching?

The symbiosis between the coral host and *Symbiodinium* (as well as the associated holobiont 'community' of microbes) is optimized under a relatively narrow window of environmental conditions of light and temperature (Drew, 1972; Hoegh-Guldberg & Smith, 1989). Consequently, a relatively small shift of one or more of these physical variables can disrupt the viability of the symbiosis (Glynn, 1990); the most conspicuous response of this disruption is coral bleaching. The term bleaching itself can be described as the apparent loss of pigmentation due to decreased numbers of their symbiotic dinoflagellates, a reduction of their photosynthetic pigments, or both (Jokiel & Coles, 1990; Suggett & Smith, 2011). Importantly, there are several types of coral bleaching (Suggett & Smith, 2011): (i) *Nonlethal*

bleaching is, and always has been, a phenomenon that effectively occurs regularly in nature as corals acclimatize to regular periodic changes in growth environment (days, seasons, etc); (ii) *Sublethal bleaching* during extreme environmental conditions whereby coral physiological processes are compromised but mortality does not occur and corals have the potential to recover once ambient environmental conditions return; (iii) *Lethal bleaching* under the most extreme or rapid environmental change where corals exhibit an irreversible 'end point' of the bleaching process to result in coral mortality.

The process of (sub)lethal bleaching, as induced by anomalous environmental conditions, can result via a range of physiological mechanisms that ultimately acts to reduce the net photosynthetic capacity of symbiotic zooxanthellae. This loss of photosynthetic capacity appears to coincide with an increased net production of reactive oxygen species (ROS), which can damage a range of cellular components, such as lipids and proteins, to induce severe physiological malfunction (Tchernov *et al.*, 2004; Suggett *et al.*, 2008). In all cases, zooxanthellae are lost from the corals' gastroderm tissue via expulsion or degradation (Gates *et al.*, 1992; Baird *et al.*, 2009) presumably, once physiological 'costs' effectively outweigh the advantages of maintaining the symbiosis. While corals can survive in the absence of their endosymbiotic dinoflagellates in the short term (Franzisket, 1970; Johannes *et al.*, 1970), the dissociation of the symbiosis results in the loss of a crucial energy source, which can have devastating impacts for the host, including the death of the coral, a reduction in reproductive output (Szmant & Gassman, 1990), and decreased rates of growth, and calcification (Glynn, 1993).

In extreme cases, (sub)lethal bleaching leads to the visible paling of the host organism, as the yellow-brown pigmentation of *Symbiodinium* is lost. In scleractinian (stony) corals, 50% or more of the total symbiont community must be lost before paling is typically

visible to the naked eye (Fitt *et al.*, 2000), and in many taxa, including corals, bleaching turns the host organism white, as the calcareous skeleton becomes visible through the coral's transparent tissues (Fig. 1-1). The coral host itself can be negatively impacted via processes such as elevated ROS production by host tissues as well as loss of host tissue adhesion from the underlying $CaCO_3$ skeleton (Downs *et al.*, 2002; Baird *et al.*, 2009). Apoptosis, triggered by the production of reactive oxygen species (ROS) in the symbiotic algae (zooxanthellae) that reside within host animal cells, can induce an apoptotic caspase cascade in the host animal that leads to expulsion of the algae and can also lead to death of animal (Tchernov *et al.*, 2011).

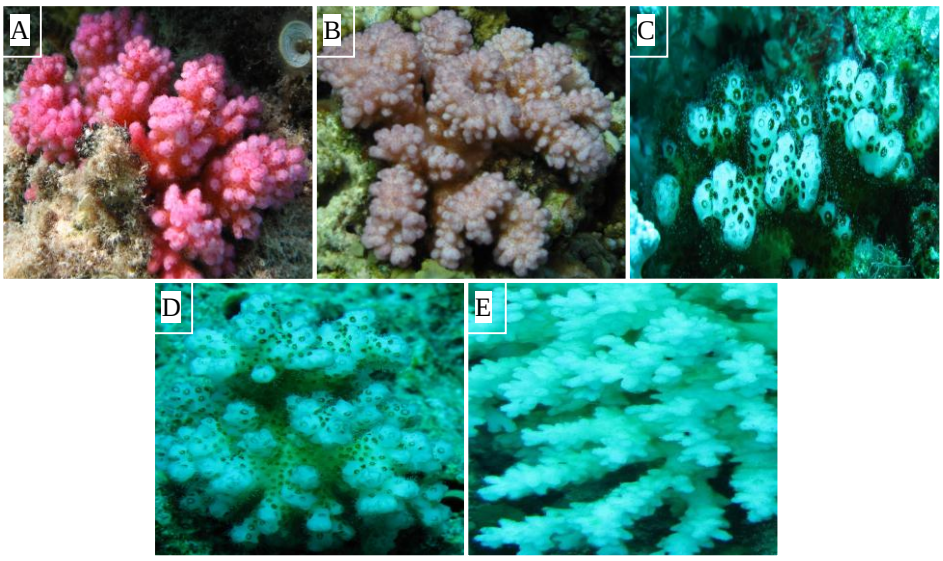

Figure **1-1**. Example of catastrophic (sub)lethal bleaching in *Pocillopora* sp (A) from the Red Sea via an initial loss of its bright color (pigments) (B) transformation to yellowish brown (C) to initial loss of color (paling and or some tissue loss) with some compensation through polyp extension during the day (probably to increase heterotrophy), (D) partially expelled zooxanthellae with obvious retracted polyps. The last step is expelling all zooxanthellae/tissue loss and becoming white in color (E).

Bleaching, the process of paling/tissue loss, likely represents a by-product in the host's normal processes of population regulation of the symbiont at high temperature. Densities of *Symbiodinium* in corals are generally low and stable (Muscatine *et al.*, 1998), despite the fact that the potential population growth rate of symbiont cells is much higher

than host cells (Falkowski *et al.*, 1993). The host must, therefore, have mechanisms that regulate symbiont densities, for example 'host factors' that limit symbiont cell division (Falkowski *et al.*, 1993) or are potentially toxic to *Symbiodinium* cells (Dunn *et al.*, 2002). However, a small (but rapid) loss in symbionts may result in a positive feedback to the host whereby the remaining cells are subject to higher light than previous and thus undergo even additional stress (Marcelino *et al.*, 2013). Such a concept paralleled recent suggestions (Cunning & Baker, 2012) that corals with inherently high symbiont densities are thus much more susceptible to bleaching.

Other host regulatory mechanisms include limiting the supply of essential nutrients to symbionts (Muscatine *et al.*, 1998) or digesting or expelling symbionts (Dunn *et al.*, 2002). At non-stressful temperatures, the majority of symbionts released appear to be morphologically degraded and have low photosynthetic efficiency (Bhagooli & Hidaka, 2004). By contrast, at stressful temperatures, the majority of released symbionts appear healthy and photo-synthetically active (Reimer, 1971). This suggests that under stress, the coral host's ability to discriminate between healthy and underperforming symbionts is diminished. Evidence for this is rare but appears consistent with additional studies examining coral bleaching and zooxanthellae dynamics (Fitt & Warner, 1995; Warner *et al.*, 1996; Ralph *et al.*, 2001).

Immunological responses may inherently contribute to this bleaching process (Palmer *et al.*, 2012). Under normal conditions, symbiosis is maintained by the release of signaling compounds from the symbiont (Yellowlees *et al.*, 2008). The host-derived symbiosome membrane can mediate this signal transduction, although the role of membrane components in inter-partner communication remains to be fully explored (Schwarz & Weis, 2003; Chen *et al.*, 2005a). When the *Symbiodinium* cells become compromised under stress, signaling is

disrupted and the animal host defends itself, by expelling the symbiont or killing the animal cells that contains the algal symbionts (Perez & Weis, 2006; Dunn *et al.*, 2007). Once anomalous temperature (or indeed light) stress subsides, corals can often recover and regain their previous levels of zooxanthellae; however, this depends on the intensity and duration of the stress (Hoegh-Guldberg, 1999b). Prolonged or extreme exposure can result in mortality of not only individual corals but also whole assemblages or reef tracts (Bellwood *et al.*, 2006; Baker *et al.*, 2008; Fitt *et al.*, 2009)

1.4. Mechanisms that lead to coral bleaching

A major focus for research over the past two decades has been to understand the primary mechanisms that drive coral bleaching and hence susceptibility to environmental changes (Smith *et al.*, 2005). The most popular working hypothesis is still the production of reduced (toxic) oxygen intermediates, Reactive Oxygen Species (ROS hereafter), in both the dinoflagellate symbiont and host tissues that subsequently causes cellular damage and expulsion of symbionts (Lesser, 1997; Smith *et al.*, 2005). ROS molecules are a by-product of photosynthesis and cellular respiration, formed by a variety of chemical, photochemical and biological pathways in a stepwise reduction of oxygen (Byczkowski & Gessner, 1988), and can play a positive role to cells; specifically used as signals for cellular defensive responses as well as apoptosis (Smith *et al.*, 2005; Lesser, 2006; Wong *et al.*, 2010), However, ROS in excess will mutate DNA, denature proteins, and oxidize lipids and cellular membranes (Lesser, 2006; Venn *et al.*, 2008). In animal cells, ROS production under normal conditions is associated with the mitochondria and peroxisomes, while in photosynthetic organisms, it is also associated with the chloroplasts (Lesser, 2006). As a result of ROS production, cells apply various strategies to mitigate their detrimental effects.

Physiological hyperoxia and exposure to UV radiation act synergistically with elevated temperatures to produce active forms of oxygen in the zooxanthellae of corals (Lesser *et al.*, 1990; Lesser, 1997; Smith *et al.*, 2005). Symbiotic cnidarians routinely experience an elevated pO_2 within their tissues as a result of photosynthetically produced oxygen (Shashar *et al.*, 1993; Kuhl *et al.*, 1995). Excessive excitation energy in the presence of oxygen leads to the production of (i) singlet oxygen ($1O_2$), typically in the antennae and photosynthetic reaction centers, which can primary target the primary photosynthetic proteins of photosystem II (PSII) (Warner *et al.*, 1999; Takahashi *et al.*, 2004, 2008); and (ii) superoxide radicals (O^{-2}); and in turn hydrogen-peroxide (H_2O_2), via electron cycling from the FeS complex at photosystem I (PSI) for which there are many cellular targets (Asada, 2006), most notably, destabilization of the thylakoid membranes (Tchernov *et al.*, 2004). Induction of excessive 'excitation energy' itself is typically the result of a loss of the photosynthetic electron transport, PET, (rather than elevated absorption of light). Thus ROS-induced damage to light harvesting and photosynthetic proteins causes a feedback loop that results in lowered PET and further potential for production of ROS (e.g. Lesser, 1997). Importantly, some evidence suggests that the enzyme ribulose1,5-bisphosphate carboxylase/oxygenase (RuBisCo) may also be targeted by heat stress ('slowing' the rate at which CO2 is fixed or direct deactivation by ROS) to contribute to a reduction in PET (Jones *et al.*, 1998; Lilley *et al.*, 2010). In all cases, the end result is a loss of photochemical activity and chlorosis (Baird *et al.*, 2009).

In order to reduce the rate of loss of photosynthetic electron transport, zooxanthellae and their host corals can employ one of two strategies: (i) accept a high rate of damage but increase the rate of repair of damaged proteins (e.g. the core D1 protein of PSII, (Ragni *et al.*, 2010; Hennige *et al.*, 2011; Hill *et al.*, 2011)) or eliminate/scavenge ROS produced by

various cellular products, such as antioxidants including catalase (CAT) and superoxide dismutase (SOD) (Asada, 2006; Merle *et al.*, 2007; McGinty *et al.*, 2012).

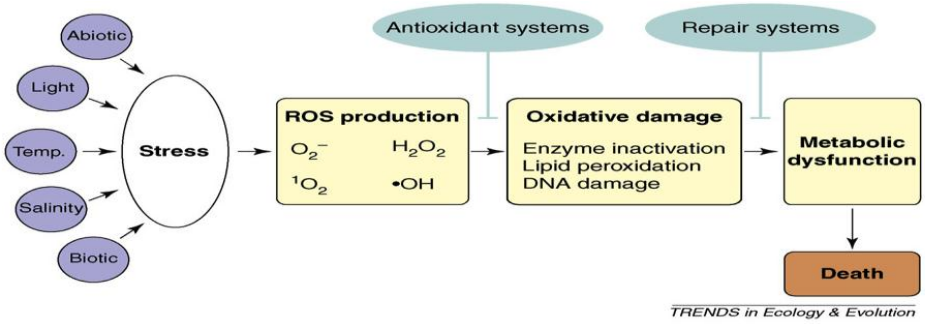

Figure **1-2**. Corals bleach in response to high temperature, high light or high salinity and so forth, and therefore bleaching can be considered a stress response. In most stress responses, the production of reactive oxygen species (ROS) in cells is a common early event such as superoxide, Hydrogen peroxide (H_2O_2), etc. Potentially toxic ROS are removed by antioxidant systems, which include enzymatic antioxidants. Under severe stress, however, these antioxidant systems might not be able to destroy all ROS produced, in which case oxidative damage will occur, leading to metabolic dysfunction, cell destruction or mutation. In addition to antioxidant systems, there are repair systems to remove damaged molecules and to replace them with new ones. In this context, antioxidant systems function as the primary line of defense and the repair systems act as a secondary line of defense against oxidative stress. If these mechanisms cannot limit or suppress stress damage, living organisms will eventually die. Diagram was adopted from Baird *et al.*(2009)

The enzymes superoxide dismutase, catalase, and ascorbate peroxidase act in concert to inactivate the effect of those toxic radicals, but as with damage-repair are 'costly' to produce and may not always be present in the abundance(s) required to scavenge the increase in the cellular flux of superoxide radicals and hydrogen peroxide (Lesser & Shick, 1989; Lesser *et al.*, 1990; Lesser, 1997). Ultimately, ROS-induced damage is not constrained to the algal cell as one of the ROS, H_2O_2, is permeable to cellular membranes, and can move out of the symbiont and into host cells, and potentially trigger the host to expel the algal symbiont (Smith *et al.*, 2005; Tchernov *et al.*, 2011).

Bleaching of the holobiont may not only be induced by the host-*Symbiodinium* association. Recent studies have proposed that bacterial pathogens can be the primary cause of bleaching in reef-building corals (Rosenberg & Ben-Haim, 2002; Rosenberg *et al.*, 2007). The Bacterial Bleaching Hypothesis (Rosenberg & Ben-Haim, 2002) arose from studies in which the bacterium, *Vibrio shiloi*, was shown to cause the annual bleaching of the Mediterranean invasive coral *Oculina patagonica* during warm summer months (Kushmaro *et al.*, 1996; Toren *et al.*, 1998). This mechanism is by the bacterial intracellular penetration of the host to induce multiplication and subsequent cellular lysis of zooxanthellae leading to annual bleaching of *O. patagonica* in temperatures above 28°C. Since then, the bacterial bleaching hypothesis has been extrapolated as an explanation for global mass coral bleaching hypothesis and proposed as an alternative to elevated sea surface temperature as the primary driver for the increase in mass coral bleaching over the past two decades (Rosenberg & Falkovitz, 2004).

An alternative hypothesis, the Coral Probiotic Hypothesis, (Reshef *et al.*, 2006) that describes the dynamic relationship between the microorganism community and environmental condition in selecting for the most competitive coral holobiont, somewhat contradicts this earlier conclusion: Ainsworth *et al.*, (2008) investigated the natural microbial ecology of *O. patagonica* during the annual bleaching and suggested that environmental stressors, such as elevated sea surface temperatures, lead to bleaching of *O. patagonica* via opportunistic colonization of the holobiont by pathogens such as *V. shiloi*.

Recently, high sequencing throughput technology (Roche 454 and Illumina) enabled research to explore deeply the microbial community associated with corals (Lee *et al.*, 2015; Ziegler *et al.*, 2015a; Röthig *et al.*, 2016; van de Water *et al.*, 2016) suggesting corals are the world's most diverse symbiotic ecosystems (Blackall *et al.*, 2015). Hernandez-Agreda *et al*

(2016) reported ca. 173 K OTUs (operational taxonomic unit) associated with *Pachyseris speciose* along depth gradient in Great Barrier Reef and coral Sea. Bayer *et al* (2013) reported high abundance of *Endozoicomonas* suggesting it appears to play an intimate role with corals. Similarly, Ainsworth *et al* (2015) identified two ubiquitous bacterial phylotypes that facilitates algal symbiosis. Consequently, there is growing evidence that microbial community plays potential roles in coral fitness and therefore, it susceptibility to bleaching, however, the functional role remains not well explored.

1.5. Deterministic factors of coral bleaching

Certain species' traits and environmental conditions regulate the rate and extent, and hence the overall susceptibility, with which corals (and other cnidarians) bleach in response to anomalous environmental conditions. In general, fast-growing branching and slow-growing massive coral species often bleach most rapidly and slowly, respectively (Marshall & Baird, 2000; Floros *et al.*, 2004; McClanahan *et al.*, 2004). McCowan *et al.*, (2012) recently analyzed 95 scientific papers reporting bleached species globally between 1982 and 2011 to support this general notion. However, the underlying reasons for this general pattern are still uncertain, but it is possible that the higher metabolic rates that would be indicative of the faster-growing species (photosynthesis and respiration) ultimately result in much greater production of ROS. Alternative explanations are that the thick tissue of slow-growing corals could provide better protection for zooxanthellae against UV-radiation and overall light stress (cited in, Grimsditch & Salm, 2006). Also, corals that grow more slowly/reproduce less often may do so because they invest much more in physiological tolerance mechanisms (Suggett & Smith, 2011). At present the underlying reason is not clear; however, more important is that many corals do not also conform to this general pattern and

arguably it is these corals that may provide the greatest explanation as to how corals can potentially genetically adapt (or invest in physiological acclimation) to anomalously warm water.

1.5.1. Environmental factors

Depending on the type and extent of the stressor(s), coral bleaching can be patchy and localized, or widespread. Localized bleaching events can be induced by several stressors, include freshwater runoff (Egana, 1982), Eutrophication and pollution (Jones & Steven, 1997), sedimentation through coastal development (Meehan & Ostrander, 1997), overfishing (Hughes, 1994), mining and physical destruction (Sebens, 1994), herbicides and pesticides (Kushmaro *et al.*, 1996), as well as increased or decreased light (Lesser *et al.*, 1990). All of these factors can act in isolation or together to cause loss of algal pigments from symbiotic invertebrates.

Light quality and quantity are important secondary factors that work in combination with temperature to moderate bleaching (Hoegh-Guldberg, 1999b). Experiments have demonstrated that increases of both PAR (400–700 nm wavelengths) (Hoegh-Guldberg, 1999a) and UV (Lesser *et al.*, 1990) can exacerbate that rate of bleaching, presumably by adding excitation pressure to an already reduced photosynthetic electron transport and overwhelming the activities of enzymes for neutralizing ROS (Shick *et al.*, 1996; Lesser, 1997). Excessive UV and PAR in nature can contribute to bleaching (e.g. Tahiti, Drollet *et al.*, 1995); consequently, patterns of bleaching responses will be influenced by factors that determine the amount of solar radiation to which corals are exposed; notably, cloud cover (Mumby *et al.*, 2001), and attenuation by particles in the water column.

Stratospheric ozone (Mumby *et al.*, 2001), and shading (Brown, 1997) by large landforms such as steep-sided shorelines (Marshall & Baird, 2000) have provided important

examples where corals that are more 'shaded' bleaching less severely: fissures (compared to summits) of massive corals and on partially shaded sides of colonies in Panama (Glynn, 1984) and Palau (West & Salm, 2003). Goreau *et al.*, (2000) reported lower bleaching mortality in very turbid waters in the Gulf of Kutch, Southwestern Sri-Lanka, Mahe, and inside the lagoon of Alphonse Atoll (Seychelles). Similarly, Mumby *et al.*, (2001b) reported an inverse depth-mortality relationship for *Porites* in French Polynesia, presumably from exponential attenuation of solar radiation with increasing depth.

Comparing coral bleaching and mortality across sites with different environmental histories has been insightful as they indicate the importance of the interaction between the environment and corals on a scale large enough to be relevant for field predictions and management priorities (Riegl & Piller, 2003; McClanahan *et al.*, 2005a, 2007a). Experimental studies show that thermal history, in addition to light history, can influence the response of reef-building corals to thermal stress (Middlebrook *et al.*, 2008; Howells *et al.*, 2013), and thermal stress may drive coral populations to develop resistance to future bleaching through selection for thermally tolerant individuals (Brown, 1997 as reviewed in Oppen *et al.*, 2009). Oliver & Palumbi (2011) investigated experimentally heat stressed *Acropora hyacinthus* collected from thermally moderate lagoon and a more thermally variable pool and reported that moderate pool corals showed nearly 50% mortality, however, variable pool corals survived well and showing low mortalities (16.6%) which are statistically indistinguishable from control.

Addition of nutrients (Nutrification) can also modify bleaching responses in a number of ways. Increases of ammonium, nitrate and phosphate can drive increased competitive success of macroalgae (Costa Jr *et al.*, 2000; Stimson *et al.*, 2001) and microalgae in reefs (Birkeland, 1987). An increased abundance of macroalgae negatively affects coral growth

and recruitment, with long-term consequences on the physical structure of the reef (Done, 1999), for example, Loya *et al.*, (2004) reported 50% coral mortality from benthic algal blooms. Importantly, the presence of macroalgae may exacerbate coral bleaching, not only through shading (Cantu *et al.*, 2008) but also through production of excessive dissolved organic matter that can over stimulate the microbial activity of corals that in turn starve the corals of oxygen (Smith *et al.*, 2006). Inorganic nutrient enrichment can further increase corals' zooxanthellae density and chlorophyll-a content (Hoegh-Guldberg & Smith, 1989; Dubinsky & Stambler, 1996) but also zooxanthellae productivity (Dubinsky, 1990) so that most photosynthetically acquired carbon is respired by the growing algae instead of being translocated to the animal (Falkowski *et al.*, 1993). The functioning of symbiosis between zooxanthellae and their host is thus disrupted. Not only is the concentration of inorganic nutrients key but the ratio of inorganic nutrients species: Phosphate limitation plays a potentially important role in the control of zooxanthellae numbers in the host tissue (Miller & Yellowlees, 1989); however, enriching zooxanthellae with just inorganic nitrogen sources can lead to phosphate starvation and ultimately a cascade of event towards bleaching-induced mortality (Wiedenmann *et al.*, 2012).

Particulate Organic Matter (POM) is easily suspended from the sea floor, reducing light for prolonged periods and reducing photosynthesis by zooxanthellae, leading to lower carbon gain, slower calcification and thinner tissues (Anthony & Hoegh-Guldberg, 2003). Moreover, elevated Dissolved Organic Matter (DOC) levels can also accelerate the growth rate of microbes living in the corals' surface mucopolysaccharide layer by an order of magnitude, suggesting that mortality occurs due to a disruption of the balance between the coral and its associated microbiota (Kline *et al.*, 2006). Sawall *et al.*, (2012) reported changes in bacterial community patterns were mostly affected by changes in nutrient availability. In

contrast, Chromophoric Dissolved Organic Matter (CDOM) in the water column can absorb UV leading to protection from bleaching (West & Salm, 2003). Due to CDOM absorbing UV radiation much more strongly than visible radiation and generally much more strongly than particulates (phytoplankton and detritus), these data indicate that CDOM may play an important role in controlling UV penetration in coastal habitats for coral assemblages. Also, POM greatly contributes to nutrient availability in many coastal regions (Furnas, 2003) and can have a nutrient content of >5%, either contained in the bacteria, phytoplankton, zooplankton and detritus (Anthony, 1999).

Much of the energy required for coral metabolism is derived from *Symbiodinium* photosynthesis; however, coral cnidarians also rely on heterotrophy to meet their energetic needs. Species that are either largely inherent upon (or can increase their reliance upon) heterotrophy survive anomalous thermal stress conditions far better than species that are largely constrained to autotrophy (Grottoli *et al.*, 2006). Seemann *et al.*, (2012) reported the ability of *Poritis furcata* to rebuild lipid stores through heterotrophy under heat stress resulted in lower mortality and strong fitness and resistance of this species especially in areas impacted by anthropogenic activities and increasing seawater temperatures. Likely, this response enables the host to compensate for the loss of energy caused by reduced densities of *Symbiodinium* cells and hence autotrophy during the bleaching process but also during recovery when environmental conditions return to ambient. Corals with large polyps rely more upon their heterotrophic abilities than those with small polyps over a variety of depths according to Muscatine *et al.*, (1989).

Low water flow has also been identified to cause localized bleaching (Nakamura & Van Woesik, 2001; Nakamura *et al.*, 2003; McClanahan *et al.*, 2005b), presumably due to accumulation of toxic free radicals in coral tissue produced by high light and temperature

(Nakamura & Van Woesik, 2001). Thus, high current speeds could actually prevent bleaching by inducing high mass transfer of detrimental photosynthetic byproducts out of the colony. Under low-flow conditions (<3 cm/second) and constant light and temperature, *Acropora digitata* suffered high bleaching mortality, whereas colonies under high flow conditions (50–70 cm/second) showed no bleaching effects (Nakamura & Van Woesik, 2001). Hence, high water flow may prevent, through diffusion, excessive buildup of toxins within corals subjected to high sea surface temperatures and high irradiance (Nakamura & Van Woesik, 2001). Anecdotal field observations in the southern Seychelles and Indonesia (see West & Salm, 2003) support this theory. Enhanced flow by upwelling can bring added benefits: whilst upwelling can reduce coral growth and potentially enhance bleaching (Glynn & D'Croz, 1990); however, localized upwelling can cool heated surface water that would otherwise bleach during regional ENSO events (West & Salm, 2003). For example, Wilkinson (2000) reported that the rapid recovery of reefs from the 1998 bleaching at north Binh Thuan, Vietnam (compared to other sites in Vietnam) was attributed to the annual upwelling at this site. Similarly, Bayraktarov *et al.*, (2012) observed in the Caribbean that when upwelling lowered the sea temperature from 28 to 21 °C, bleaching was less prevalent and recovered faster.

1.5.2. Enhanced physiological tolerance

Coral susceptibility to stress and bleaching is highly variable both within and between reef systems (Coles & Brown, 2003) suggesting some potential for corals and their endosymbionts to adapt or acclimate to warming ocean temperatures (Douglas, 2003). Three general processes can regulate the thermal tolerances of corals and their endosymbionts; (1) Adaptation via natural selection for heat-tolerant lineages of the coral host; (2) *Symbiodinium* adaptation via natural selection for heat-tolerant lineages of the algal endosymbiont; and (3)

Physiological acclimatization to the changing conditions by living individuals of either or both partners (Gates & Edmunds, 1999; Edmunds & Gates, 2008; Weis, 2010). It is important to distinguish between adaptation, which is defined as changes in the genetic structure of a population/species (usually the result or natural selection or migration), and acclimatization, which can be defined as long-term phenotypic (physiological and/or behavioral) changes that result adjustment of the organism's tolerance levels (Coles & Brown, 2003).

Most zooxanthellae were considered to be members of a single pandemic species, *Symbiodinium microadriaticum*. Pioneering studies by Trench (Trench, 1979; Schoenberg & Trench, 1980a, 1980b) and Rowan (Rowan & Powers, 1991, 1992) revealed that zooxanthellae were a highly diverse group of organisms which may include hundreds of taxa with perhaps as many as two or three species per host in some invertebrate species (Rowan *et al.*, 1997; Loh *et al.*, 1998).

Trench & Blank (1987) further provided strong morphological and biochemical (isozyme analysis) evidence for the existence of different zooxanthellae taxa associated with specific coral hosts. These initial observations have recently been supported by molecular analysis of the small subunit ribosomal RNA gene, which revealed four major groupings or clades of *Symbiodinium* designated A, B and C and D, (Baker, 2003). The distinction between zooxanthella genotypes is based on different parts of the zooxanthella genome such as nuclear genes encoding either the small subunit (SSU) or the large subunit (LSU) of the ribosomal RNA, internal transcribed spacers (ITS1 and ITS2), 5.8S regions and large subunit chloroplast rDNA (cprDNA). So far, the various genome regions have yielded a relatively robust division of zooxanthellae into 9 clades (A–I) and additional subclade (Fig. 1-3), based on nuclear ribosomal DNA and chloroplast DNA with each clade containing many species according to Pochon & Gates (2010).

Each "clade" contains multiple genetic varieties often resolved using the internal transcribed spacer (ITS) regions (van Oppen *et al.*, 2001; Pochon *et al.*, 2007). The nuclear internal transcribed spacer region 2 (ITS2) is currently most often utilized to resolve *Symbiodinium* diversity within the phylogenetic clades A–I (LaJeunesse *et al.*, 2003; Stat *et al.*, 2009), and is being promoted as a species level marker (LaJeunesse, 2001; LaJeunesse & Thornhill, 2011). Sampayo *et al.*, (2007) suggest that small differences in the ITS2 region may indeed confer functionality as ITS2-types are regulated on a fine scale in relation to local environment. However, the multi-copy nature and intragenomic variability of the ITS2 (Thornhill *et al.*, 2007) often results in the isolation of more than one ITS2 sequence type from an individual *Symbiodinium* cell, and this interpretational complexity combined with low genetic divergence among ITS2 sequences (LaJeunesse *et al.*, 2005) makes the application of this marker in species assignment problematic (Correa & Baker, 2009; Stat *et al.*, 2009). Lajeunesse *et al.*, (2012) has coupled such molecular-based approaches into a pioneering integrative evolutionary genetics approach such that *Symbiodinium* strains can potentially be assigned to specific species: in this case *Symbiodinium minutum* is harbored by widespread tropical anemones in the genus *Aiptasia* whereas *Symbiodinium psygmophilum* is harbored by subtropical and temperate stony corals (e.g., *Astrangia*, *Cladocora*, and *Oculina*).

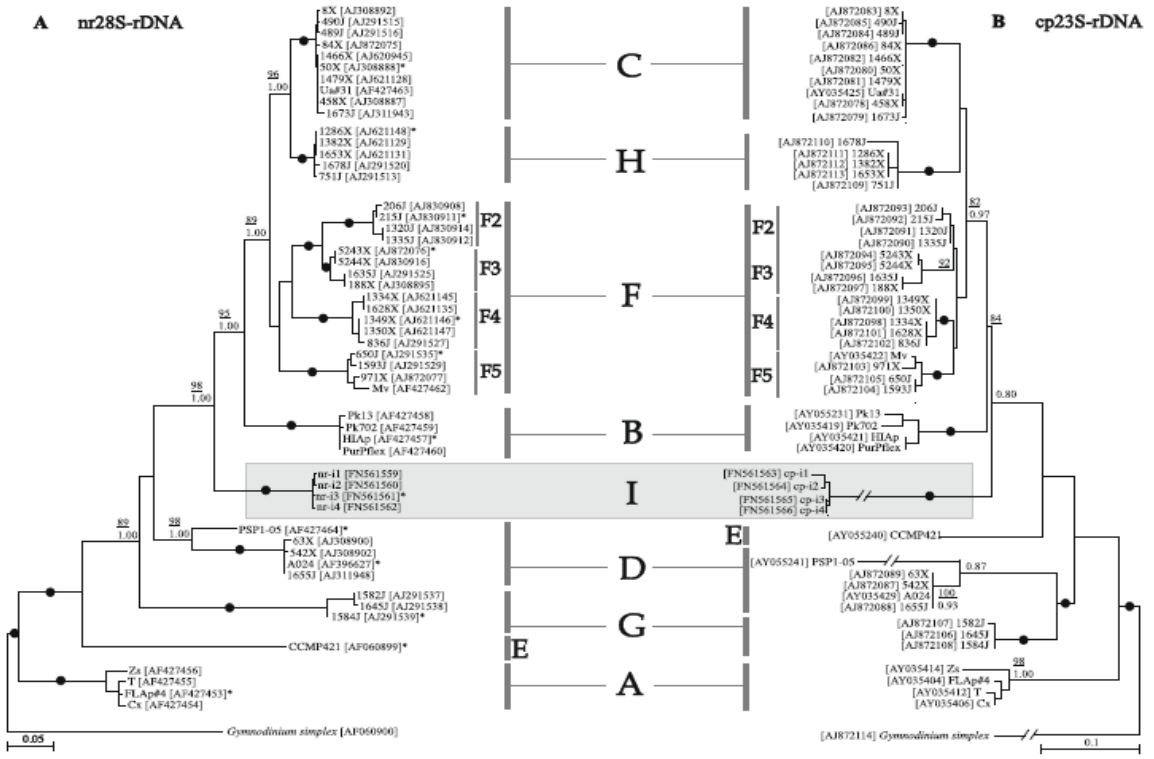

Figure **1-3**. Maximum likelihood phylograms of the genus *Symbiodinium* showing the position of clade I based on the previously published (A) nr28S and (B) cp23S datasets (Pochon *et al.*, 2006). Numbers at nodes represent the result of the ML bootstrap analysis (underlined numbers; hundred bootstrap pseudoreplicates performed) and Bayesian posterior probabilities. Black dots represent values of 100% bootstrap support (BP) and Bayesian posterior probabilities (BiPP) of 1.0. Nodes without numbers correspond to supports weaker than 70% BP and 0.8 BiPP. For each sequence, a sample reference number is indicated, followed by the corresponding GenBank accession number in square brackets. The 13 nr28S sequences selected for the genetic divergence analysis are indicated by asterisks.

It is well established that tolerance to heat (and light) stress differs amongst *Symbiodinium* clades (and importantly subclades, e.g. Robison & Warner, 2006), presumably via adaptive differences in acquisition of mechanisms used to protect against these stresses. Genetically different types of *Symbiodinium* also exhibit distinct physiologies, some of which may mitigate the effects of coral bleaching conditions (Rowan, 2004). For example, Iglesias-Prieto & Trench (1997) suggested that *Symbiodinium corculorum* (Clade A) has limited photo-acclimation capability, however, Muller-Parker (1987) found that *S. muscatinei* and *S. californium* (Clade B) are tolerant of high light intensity. Stat & Gates (2011) suggested that clade D of *Symbiodinium* are thermally tolerant coral endosymbionts that confer resistance

to elevated sea surface temperature and bleaching to the host and thus clade D has been considered "a nugget of hope" for corals to withstand future elevations in sea surface temperature. In all of these examples, the host naturally associates with zooxanthellae from several clades to result in functionally different holobionts (termed 'ecospecies') (Buddemeier *et al.*, 2004).

Numerous studies have now identified that corals can 'host' more heat tolerant *Symbiodinium* subclades during or after an anomalous stress event (Rowan *et al.*, 1997; Baker, 2001; Baker *et al.*, 2004; Berkelmans & van Oppen, 2006) or indeed when existing in 'extreme' long term environments, such as shallow rock pools (Oliver & Palumbi, 2011a). In fact, according to the adaptive bleaching hypothesis, corals can respond to thermal stress by shifting to symbioses with more temperature tolerant species of *Symbiodinium* (Brown *et al.*, 2002; Baker *et al.*, 2004; Rowan, 2004); here the algae may enter the symbiosis from exogenous sources (symbiont 'switching', Baker, 2003) or, if multiple zooxanthellae already concurrently exist within the host, a shift in symbiont dominance may occur (symbiont 'shuffling') (Berkelmans & van Oppen, 2006); such shuffling may occur during anomalous events but also regularly in response to seasonal changes in the environment (Chen *et al.*, 2005b). Intriguingly, recent evidence from the Great Barrier Reef has demonstrated how a *Symbiodinium* sub-phylotype (C1) has adapted to tolerate different temperature regimes (Howells *et al.*, 2013). Recently, Boulotte *et al* (2016) provided an evidence that coral host can switch *Symbiodinium* to more thermally tolerant clade type (clade D) suggesting high flexibility of host-symbiont symbiosis.

Regardless, it is currently unclear whether 'switching' or 'shuffling' is primarily a persistent or temporary phenomenon. An adaptive shift in symbiont communities would indicate that reefs could be more resistant to future thermal stress, resulting in significantly

longer extinction times for surviving corals, than had been previously assumed (Baker *et al.*, 2004). However, there is much debate as to whether hosting more thermally tolerant strains comes at a cost to the host (less efficient nutrient transfer from the symbiont to the host) and that corals will inherently 'defer' back to thermally sensitive symbionts once 'business as usual' environmental conditions return (Lesser *et al.*, 2013).

Although the evidence for a primary role of the symbiont in coral bleaching is overwhelming, there are many ways in which the cnidarians host can also limit the level of damage sustained by the symbionts, and thus influence the bleaching susceptibility of the holobiont (Baird *et al.*, 2009; Bellantuono *et al.*, 2012a). Barshis *et al.*, (2010) showed genetic differentiation in host populations suggesting strong selection for physiological adaptation to differing environments across small geographic distances.

Fluorescent pigments- Green fluorescent proteins (FPs) belonging to a single family of proteins (Mazel, 1995; Salih *et al.*, 2000) and specific chromoproteins (Dove *et al.*, 2006; Smith *et al.*, 2013) are located within the tissue of the host in vesicles above *Symbiodinium* cells in high-light environments; various bio-optical research suggests a photo-protective role for the symbionts (Salih *et al.*, 2000; Smith *et al.*, 2013). FPs are highly abundant on reefs; up to 97% of corals in shallow water on the Great Barrier Reef contain fluorescent pigments (Salih *et al.*, 1998, 2000). By absorbing, scattering and dissipating high-energy solar radiation via fluorescence proteins, FPs reduce photo-inhibition and the severity of bleaching damage to corals (Salih *et al.*, 2000). Smith *et al.*, (2013) provided a direct evidence to support a photo-protective role of the non-fluorescent chromoproteins (CPs) that form a biochemically and photo-physically distinct group of GFP-like proteins during investigation of *Acropora nobilis* in Great Barrier Reef.

Many aspects of bleaching appear to be related to differences in the abundance of FPs. For example, bleaching susceptible taxa, such as Pocilloporids and Acroporids, have relatively low densities of FPs, whereas poritids, faviids and other less-susceptible taxa have relatively high densities of FPs (Salih *et al.*, 2000). Similarly, highly fluorescent colonies suffer less partial mortality following bleaching than weakly fluorescent conspecifics (Salih *et al.*, 2006). Although the support for a role of FPs in reducing bleaching damage is compelling, a direct role of FPs in preventing bleaching, for example by providing photo-protection, remains to be tested experimentally. Furthermore, it is not known whether FPs can provide relief from heat stress alone. Palmer *et al.*, (2009) documented H_2O_2 scavenging of scleractinian FPs both *in vivo* across multiple species and *in vitro* with purified proteins which support the role of FPs in coral stress and immune responses and highlight the multi-functionality of these conspicuous proteins. In molecular scale, Seneca *et al.*, (2010) showed up-regulation of gene expression for the blue chromoprotein gene *Am*Ch identified in field bleached colonies, however, Smith-Keune & Dove (2008) showed the rapid down-regulation of another GFP-like gene *Am*A1 (a homologue of the red fluorescent *Zoanthus* sp. protein zoan2RFP) in laboratory heat stressed *A. millepora* colonies experiencing temperatures of 32 and 33°C.

Mycosporine-like Amino Acids MAAs- Zooxanthellae clades are able to protect themselves from ultraviolet radiation by production of MAAs by dissipating UV energy as heat without forming toxic intermediates (Shick & Dunlap, 2002); otherwise, absorption of UV can enhance production of ROS that is a key in the bleaching process (Lesser, 2006). Some differences in the tolerance of hosts and symbionts may be attributable to different kinds and concentrations of MAAs produced by specific zooxanthellae, and differences in

MAA biochemistry can be found in host tissues (Banaszak *et al.*, 2000, 2006). MAAs are synthesized via the shikimic acid pathway and, because animals lack this pathway, symbionts are presumed to be the source of MAAs in corals (Shick & Dunlap, 2002). Alternatively, they are acquired via heterotrophy (Grottoli *et al.*, 2006). Whatever the source of these compounds, MAAs are far more abundant in host tissues than in freshly isolated symbionts (Shick & Dunlap, 2002). In addition, the diversity of MAAs found in holobionts is far greater than that found in *Symbiodinium* in isolation (Shick & Dunlap, 2002). Whether this results from the host stimulating symbionts to produce a greater diversity of MAAs *in hospite* or because the host can modify MAAs translocated from the symbiont remains unknown (Furla *et al.*, 2005). However, it is clear that the host has a major influence on the complement and distribution of MAAs in the holobiont, thereby moderating the amount of UV that reaches symbiont cells.

Heat-shock proteins- Proteins, such as the ubiquitous heat-shock proteins (HSPs), act as molecular chaperones, which maintain protein structure and cell function, particularly following stress (Arya *et al.*, 2007). Many different HSPs are found in coral tissue and their activity influences the bleaching response. For example, high-light acclimatized tissues of the coral *Goniastrea aspera* have higher concentrations of HSPs and these tissues do not bleach, unlike areas of the same colony that had not acclimatized to high-light (Brown *et al.*, 2002). Also, host HSP70 protein level increased in two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata* after exposure to short-term thermal stress (Fitt *et al.*, 2009). Importantly, neither the clade of symbiont nor algal antioxidant defenses varied within colonies, highlighting the role of host tissue in the bleaching response of these colonies (Brown *et al.*, 2002). Buckley & Hofmann (2004) stated that the mRNA encoding HSP70

protein is typically produced at elevated quantities after exposure to thermal stress. Also, Gehring & Wehner (1995) and Bedulina *et al.* (2010) reported that HSP70 often has higher constitutive gene expression levels in more thermally tolerant populations or populations from more thermally extreme habitats, as has been shown in a diversity of metazoan taxa including crustaceans, insects, echinoderms, and mollusks.

Antioxidant systems- Potentially toxic ROS caused by stress are removed by antioxidant systems, which include enzymatic antioxidants such as super-oxide-dismutase (SOD) and catalase, ascorbic acid, carotenoids (Lesser, 2006) and mycosporine glycine (Yakovleva et al. 2004). Hosts, such as the coral *Stylophora pistillata* and the anemone *Anemoniaviridis*, have many different types of SOD, some of which are not found in non-symbiotic animals, and these act in combination with the antioxidant defenses (Yakovleva *et al.*, 2004) of the symbiont to minimize oxidative damage (Richier *et al.*, 2005). Higuchi *et al.* (2008) observed for *Galaxea fascicularis* during elevated seawater temperature experiments that both SOD and CAT activities in coral tissue and zooxanthellae were increased. More types of SOD are active in symbionts in isolation than when *in hospite*, indicating that protective mechanisms of the host limit oxidative damage sustained by the symbionts (Richier *et al.*, 2005).

Changing ROS concentrations results activation or silencing of genes encoding for the various defensive antioxidants (Dalton *et al.*, 1999). For Example, Seneca *et al.* (2010) have found a significant up-regulation of catalase genes in *A. millepora* during natural coral bleaching events thereby supporting previous studies indicating that oxidative stress likely affects host coral tissue during bleaching events (Lesser, 1997, 2006; Lesser & Farrell, 2004). In fact, expression of antioxidants is now being used to assay stress sensitivity: McGinty *et*

al., (2012) measured the production of ROS (at 26 °C, 29 °C, 30 °C, and 31°C) and activity of the antioxidants catalase (CAT) and superoxide dismutase (SOD) (at 26 °C and 31 °C) of seven genetically distinct types of *Symbiodinium*, including A1, B1, B2,C1, D, E1, and F2, demonstrating that the relative heat tolerance of these species was in fact related to antioxidant expression. Such studies thus further confirm that establishing symbiont identity is critical when exploring the response of intact associations to this type of stress.

All physiological, genetic and environmental factors mentioned above may act separately or synergistically to drive corals to be thermally tolerant or susceptible for global warming. Despite this, corals inhabit extreme/marginal systems appear thermal tolerance more than usual, and hence get high attention as they survive in SST predicted for the future. Researchers consider extreme systems are as a "natural laboratory" to understand the underling mechanisms of corals' adaptation/acclimation.

1.6. The Red Sea as a hot spot for warm waters

The Red Sea is a unique basin of semi-enclosed water separating northeast Africa from the Arabian Peninsula and is thus semi-isolated from neighboring larger bodies of water (Fig. 1-4). It lays between 30°N and 12°30`N and spans nearly 2000 Km of navigable water in length (~280 Km in width) and is forked at the north to form the Gulfs of Suez and Aqaba. The Red Sea is linked to the Indian Ocean through Gulf of Adan via the Strait of Bab el Mandeb and to the Mediterranean via the Suez Canal, to form a vital route for world trade between Europe and America. It is surrounded by a narrow coastal fringe (~40-60 km wide) and backed by dry and largely arid semi-desert hills or mountains, which can rise to 3,000 m (Berman *et al.*, 2003).

1.6.1. Oceanography

The geographical position of the Red Sea results in extreme climatic conditions so that it is one of the warmest and most saline water basins with a direct connection with the ocean (Sofianos *et al.*, 2002). Evaporation in the Red Sea averages 1–2 m yr^{-1} in both summer and winter and it receives little freshwater input (30 mm $year^{-1}$). Such extreme loss of water results in a gradual increase in salinity towards the north. For example, Salinity at Bab El Mandeb averages 37‰, due to water influx coming from Gulf of Adan, but increases northward at the entrance to the Gulfs of Aqaba and Suez to ~41-42‰ (Edwards & Head, 1986).

Rain over the Red Sea is very sparse and localized with many areas not receiving any rain for months or years. In both Gulfs of Aqaba and Suez, rainfall is typically <25 mm/yr and is confined almost entirely to the period September to March (Edwards & Head, 1986). The western shore, from Hurghada south to about 22°N, is virtually rainless often amounting to only a few millimeters over several years (Fouda *et al.*, 1994).

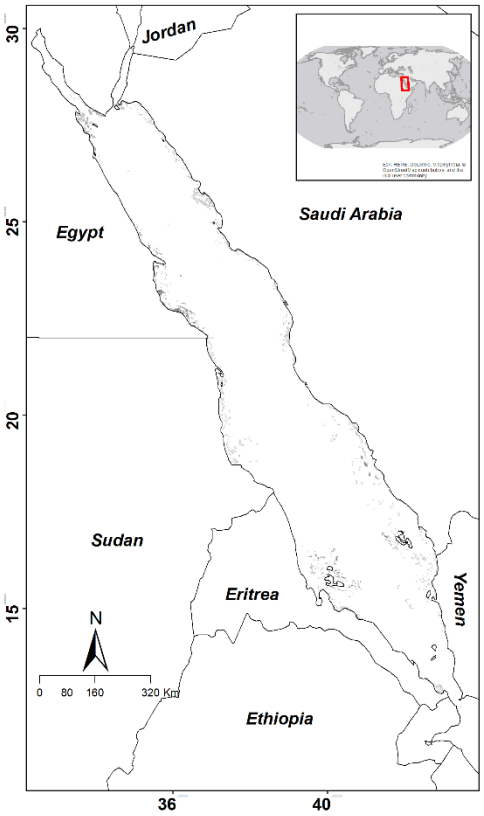

Figure **1-4**. Red Sea map shows its geographical location and the semi-enclosed nature and connections with Indian Ocean through Gulf of Adan and Mediterranean Sea through Suez Canal.

Annual variations in sea surface temperature are in part controlled by annual wind patterns but also density gradients: Inflowing surface water during winter becomes denser as it flows northward via evaporation (increased salinity) and cooling thus sinking towards the north Suez Gulf entrance; this sinking causes intermediate water inside the basin to ascend and flow out over

the sill of Bab El-Mandab (Fouda *et al.*, 1994). Sea surface temperatures (SSTs) in winter (February) range from 18 and 21°C in the northern and southern Gulf of Suez, respectively, and increase to a maximum of 26.5°C at the southern end of the Red Sea (~17° 30` N). Mean SSTs in summer time (August) are ~27°C in the Gulf of Suez and ~31.5°C in the southern half of the Red Sea between Yemen and Eritrea (Edwards & Head, 1986). The combination of high/stable temperatures and low rainfall/runoff result in high transparency of the waters (e.g. 77–105 m in the Gulf of Aqaba and 74–94 m in the northern Red Sea, Stambler, (2005), with permanent stratification and a strong thermocline. Thus waters are often oligotrophic, limited to low new nutrient inputs and generally very low primary productivity (Longhurst, 1998).

The prevailing winds over the Red Sea run along the north-south axis throughout the year, except in the south where the direction reverses in winter (Edwards & Head, 1986). Wind direction changes from offshore during the day to onshore at night driven by differential heating and cooling of land and sea. As such, the predominant water currents follow the prevailing wind patterns. Tides in the Red Sea are predominantly semi-diurnal with different ranges changes from north to south: ~1.5-1.8 m in the north (Suez Gulf) but only ~0.90 m from the entrance of both Aqaba and Suez Gulfs to the south (Fouda *et al.*, 1994). Overall, the semi-enclosed nature of the Red Sea limits the turnover of the water body to ~200 years (Sheppard *et al.*, 1992).

1.6.2. Coral reefs of the Red Sea

The Red Sea is a globally significant marine ecosystem, renowned for unique marine and coastal environments and high species richness including many endemic species; it contains representatives of all major tropical marine ecosystems except estuaries (PERSGA, 2010). Coral reefs represent the most distinct ecosystem in the Red Sea and provide a

valuable source of income via tourism, estimated to be >0.5M people annually from all over the world; this tourism continues to grow and provide demand for tourism infrastructure and delivering important foreign revenue to the regional and national economy (USAID, 2012). Red Sea reefs are amongst the best-developed reefs in the western Indian Ocean (PERSGA/GEF, 2003), primarily as fringing reefs around the mainland and coastal islands and to a lesser extent as lagoons, barrier reefs, pinnacles, and atolls (PERSGA/GEF, 2003). Here, reefs are characterized by relative high diversity of ~250 and ~180 species of hard (hexacoralia) and soft (octocoralia) coral species and an even higher diversity of fish (Sheppard *et al.*, 1992); although more recent studies have documented upwards of 260 (Devantier *et al.*, 2000) to 300 species of hard corals (PERSGA, 2006; Wilkinson, 2008) as a result of recently described species via range extensions.

Northern and central Red Sea reefs are the best developed with reef complexes all along the coast at about 3-10km offshore, formed via a series of narrow underwater banks of tectonic origin. Here, hard coral diversity ranges from 143 species (central Red Sea), 130 species (Gulf of Aqaba) and 128 species (northern Red Sea). This northerly reduction in diversity appears to reflect the decrease in the quality, complexity and extent of reefs due to shallower bathymetry, higher turbidity, and greater freshwater input South of 20^{o} N (Edwards & Head, 1986). The species composition of soft corals comprise 28 species belonging to five genera *Rhytisma, Sinularia, Paralemnalia, Scleronephthya*, and *Heteroxenia* (Benayahu *et al.*, 2002).

Several general patterns of coral distribution can be summarized as follows: Hard corals show a decrease of percentage cover with increasing depth (Kotb, 1989); in contrast, soft corals exhibit an increase in cover with increasing depth from 0-15 m but decrease with depth below 15m (Kotb, 1989). Branched and massive corals dominate the reef crest and

encrusting ones are abundant in deeper waters (Edwards & Head, 1986). Zonation is typically characterized by species of *Acropora* in the shallow fore reef, followed by species of *Millipora* down to 10 m and species of *Montipora* to 20-25m (but gradually replaced by soft corals from10- 40m). The most common hard corals include *Favia, Favites, Echinopora, Porites, Pocillopora, Fungia, Pavona, Gardineroseris and Dendrophyllia (*Fouda & Gerges, 1994), whilst the most common soft corals are *Sinularia, Dendronephthy* and, Xeniidae (Benayahu *et al.*, 2002).

1.7. Bleaching in the Red Sea

1.7.1. Patterns of Bleaching

Red Sea corals effectively already exist in "future thermal environments" predicted for tropical reefs worldwide for the next 50 years. High variability of SSTs along the central coast of the Red Sea exhibits a seasonal range of ~10 °C with summer temperatures often exceeding 32°C (Davis *et al.*, 2011). Most corals would currently perish under such conditions thereby providing an insight into the present limits of holobiont adaptation (Riegl *et al.*, 2011) and the mechanisms/processes required to thrive in a future warmer ocean (Jokiel & Coles, 1990; Palumbi *et al.*, 2008; Oliver & Palumbi, 2011a). That is not to say that Red Sea corals will be unresponsive to changing SSTs: Cantin *et al.* (2010) have recently shown that rising SSTs have already driving changes in the growth of an important reef-building coral in the central Red Sea; SSTs have already increased by ~0.7°C since the last decade (Raitsos *et al.*, 2011) and are predicted to rise by a further 2.5-3°C relative to the 2000–2008 mean by 2099.

Mass coral bleaching has been observed with increasing frequency since the 1980s for many coral reefs. After this global event, Red Sea has not shown mass mortality except some localized bleaching summarized in Table 1-1. The northern Red Sea has remained

relatively unaffected by these temperature-induced bleaching events (Sotka & Thacker, 2005). Heiss *et al.* (1999) showed that sea surface temperatures in the northern Gulf of Aqaba had increased by at least 1.3°C since the early nineteenth century. As a possible consequence of these elevated temperatures Loya (2004) described for the first time sporadic coral bleaching during the summers of 2002 and 2003 but appears to have thus far escaped a major mass bleaching event (Rouphael & Abdulla, 2007). Similarly, the Gulf of Aqaba is characterized by extreme environmental conditions such as catastrophic low tides, elevated temperatures and high irradiance (Yossi, 1986; Achituv & Dubinsky, 1990) however; no bleaching events have been reported from this area (Pilcher & Alsuhaibany, 2000). PERSGA (2009) has not reported any mass bleaching events along the Red Sea, and all recorded bleaching has been very localized and likely induced by direct influence of anthropogenic activities and within the normal natural mortality.

Table **1-1.** Summary of bleaching pattern in the Red Sea during last decade

Country	year	Description	Reference
Egypt	2007	Two events: (i) Extreme low tides in March exposed reef flats from the Gulf of Aqaba to the Fury shoals, 430 km south of the Sinai Peninsula, resulting in extensive coral bleaching and mortality. (ii) Warm water bleaching event in October, with major coral bleaching to 20 m depth at 'Rocky Island', 450 km south of the Sinai Peninsula. Bleaching followed the September predictions from NOAA of a 'hot spot' in the central Red Sea based on Degree Heating Week (DHW) analyses. Extent of coral mortality not quantified.	(Wilkinson, 2008)
	2012	Bleaching has been recorded when DHW was high and SST anomalies according to NOAA Bleaching watch which is considered the primary causative factor. Bleaching average was 14.5 % of total coral cover ranged from 10 % in 4 surveyed sites to 20% in only three sites of 10 surveyed sites.	(HEPCA, 2012)
Saudi Arabia	1998	Bleached occurred when sea surface temperatures exceeded 31ºC, which was more than 2°C above the mean monthly average. About 10% of reefs between Jeddah and the Gulf of Aqaba showed evidence of bleaching; however, it was intense near Rabigh, with recently dead and bleached coral accounting for up to 90% of total cover on shallow reef slopes. On affected reefs, bleaching was most intense in depths less than 6 m but it	(Devantier & Pilcher, 2000)

		was also observed below 20 m. Coral cover in the worst affected communities should recover in 1–2 decades.	
	2010	Coral bleaching event occurred in the central Red Sea near Thuwal, Saudi Arabia, in the summer of 2010, when the region experienced up to 10–11degree heating weeks. Resurveying has been conducted on the reefs 7 months later to estimate subsequent mortality and documented the susceptibility of various coral taxa to bleaching at eight reefs during the peak of this thermal stress. Shallow reefs and inshore reefs had a higher prevalence of bleaching. Significant factors in the likelihood of coral bleaching during this event were depth of the reef and distance of the reef from shore.	(Furby *et al.*, 2013)
Eritrea	1998	SST reached 40°C and bleaching occurred on shallow and deep reefs however, most corals recovered after the temperatures dropped	(Wilkinson, 1998)
Yamen	1998	Significant bleaching occurred after SST increased by about 2 ºC. However, the onset of the south-west monsoonal upwelling rapidly cooled this in July. Bleaching-associated mortality was particularly significant at Belhaf and Hadhramaut reefs. In April 1998 live and dead coral coverage was, respectively, 51–75% and 1–10% at Belhaf, and 31–50% and 1–10% at Hadhramaut. In December1998 live and dead coral coverage was, respectively, 11–30% and 31–50% at Belhaf, and 11–30% and 11–30% at Hadhramaut	(DeVantier & Hariri, 2000)
Sudan	1998	Poor cover of living coral in 2002 in the Mukawwar Island and Dungonab Bay MPA outside of Dungonab Bay has attributed to 1998 bleaching event. Corals in the Dongonab Bay showed patchy mortality; in some areas, it was 90% from 0 – 15m depth, while other areas were almost entirely unaffected. Coral recovery has been patchy: some areas show high levels of recruitment and growth, but many others show no recovery.	(PERSGA/GEF, 2003)

Species Susceptibility- The most comprehensive survey of bleaching susceptibility amongst coral taxa to date for the Red Sea was by Furby *et al.* (2013). Here, reefs were surveyed pre and post-summer 2010 of a major localized coral bleaching event near Thuwal, Saudi Arabia. Oculinids and agaricids were most susceptible to bleaching, with up to 100 and 80 % of colonies of these families, respectively, bleaching at some reefs. In contrast, some families, such as mussids, pocilloporids and pectinids showed relatively low levels of bleaching (<20 % on average). Mortality was highly variable among taxa, with some taxa showing evidence of full recovery and some (e.g. Acroporids) apparently suffering nearly complete mortality. The unequal mortality among families resulted in a significant change to

the community composition following the bleaching. Southern part Egyptian Red Sea water showed mass bleaching affected mainly *Porites* sp, *Millepora* sp and *Montipora* sp, while affected moderately *Acropora* sp, *Stylophora* sp, *Pocellipora* sp and the massive corals *Favites* sp and *Galaxia* sp as well as soft coral genus *Sinularia* according to HEPCA (2012). Red Sea reefs are not immune to increasing global pressures and the rising SSTs may be approaching the thermal tolerance limits of the corals. Kelman *et al.* (2006) stated that 83% of Red Sea alcyonacean soft corals exhibited appreciable antimicrobial activity against marine bacteria isolated from the seawater surrounding the corals, while the stony corals had little or no antimicrobial activity which may interpret the susceptibility of hard corals.

1.7.2. Mechanisms of bleaching in the Red Sea

Relatively few studies have considered the underlying mechanisms driving the bleaching patterns and certainly none have been conducted during the few bleaching events that have occurred. Most to date from the Red Sea comes from a very limited area (primarily Eilat, e.g. Spaet *et al.* (2012)), with comparatively little information from the central and southern Red Sea. There is an almost complete lack of empirical data documenting the effects of thermal stress in this region limits our ability to predict how future increases in SST will shape the coral reef landscape of the central Red Sea over the next century (Cantin *et al.*, 2010).

Previous studies in the Red Sea have recorded bleaching due to transient freshwater runoff in the warm season (Antonius, 1988). Much of the focus of bleaching mechanisms in the Red Sea has been on microbial (Vibrio)-based bleaching. Ben-Haim (2003) reported that bleaching of the coral *Pocillopora damicornis* on the coral reefs in the Red Sea is the result of an infection with *Vibrio coralliilyticus*. Furthermore, Ritchie & Smith (2004) demonstrated that *Vibrio* populations increased during bleaching and returned to previous

levels during recovery. The bacterial community of *O. patagonica* naturally changes with the seasons (Koren & Rosenberg, 2006); however, infection and resultant coral bleaching only occurred at elevated seawater temperatures above the seasonal ambient (Kushmaro *et al.*, 1998). The first step in the infectious process is the adhesion of *V. shiloi* to a β-galactoside containing receptor on the coral surface (Toren *et al.*, 1998) with temperature of bacterial growth critical for the adhesion of *V. shiloi* to the coral. Bacteria grown at the elevated seawater temperature adhere avidly to corals maintained at either low or high temperature. Importantly here is that the environmental stress condition was causing the coral bleaching pathogen to express its virulence (Banin *et al.*, 2000). Recently, Armoza-Zvuloni *et al.* (2011) discovered the occurrence of unusual multifocal lesions, presumably bleaching, on *Millepora dichotoma* populations in Red Sea, Eilat. In an attempt to identify the microbial community associated with the affected tissue, Paramasivam *et al.* (2013) isolated strain MD2^T from the affected tissue and used 16S rRNA gene sequence analysis to reveal similarity to strains from of the genus *Kordiimonas* which were already isolated from the marine environment. Recently, Jessen *et al.* (2013) reported distinct bacterial species, a consequence of eutrophication and/or, overfishing, belonging to the genus *Endozoicomonas* consistently abundant and constituted two-thirds of bacteria in the species *Acropora hemprichii*. Thus the underlying microbial microflora of the coral holobiont clearly displays important signatures in relation to environmental perturbations but whether this is a cause or a consequence of the bleaching susceptibility is still undetermined.

1.7.3. Coral resistance

Few studies to date have examined, let alone identified, components that may be integral to defining inter-species susceptibility to thermal stress amongst coral taxa.

Heat shock proteins—Tom *et al.* (1999) examined heat shock protein 70 gene (HSP70) from *Stylophora pistillata* as a tool for thermal stress susceptibility and confirmed that HSP70 mRNA expression in corals grown within their normal physiological conditions; this protein has been shown to belong to the coral genome and not to its symbiotic algae one. Wiens *et al.* (2000) investigated the expression of the HSP90 gene in the Red Sea octocoral *Dendronephthya klunzingeri* heated experimentally (2 h at 4 °C above ambient temperature) as well as for *in situ* specimens exposed to natural thermal stress using cDNA, and reported up-regulation of HSP90 during thermal stress in all cases. Finally, Chow *et al.* (2009) investigated HSP60 in *Stylophora pistillata* (branching) and *Turbinaria reniformis* (laminar), which exhibit marked differences in their ability to survive in stressful environmental conditions. HSP60 induction was observed in the laminar coral following either light or thermal stress whilst the branched coral exhibited comparatively weak transient HSP60 induction after heat stress and no detectable induction following light stress; these results were indeed consistent with their relative susceptibility to bleaching *in situ*.

Symbiodinium genotypes—Baker *et al.* (2005) used molecular DNA techniques to identify and compare zooxanthellae of reef corals from a site in the Arabian Gulf that experienced severe bleaching and mortality, and two sites in the Red Sea that experienced little or no bleaching or mortality. Corals in the Red Sea contained mainly *Symbiodinium* C with some A and occasional D, whilst those from the Arabian Gulf were dominated by *Symbiodinium* D (86.2%), with some C (10.3%) and occasional A. Similarly, Barneah *et al* (2004). reported that Red Sea octocorals, collected from Israel, hosted predominantly

Symbiodinium C, with *Symbiodinium* A occurring in 3 species, *Litophyton arboretum*, *Nephthea* sp and *Stereonephthya cundabiluensis*, of 19 studied species.

In contrast, more is known of *Symbiodinium* genotype diversity and association from the Gulf of Aqaba (Eilat): Barneah *et al.* (2004) found for the first time that all hosts using horizontal transmission harbored symbionts belonging to clade C, while those with vertical transmission uniquely harbored symbionts from clade A. The limitation of clade A symbiont to hosts with vertical transmission suggests a co-evolution of the hosts and symbionts, while clade C symbionts, characterized by large sub-clade variability, genotypes exhibit more specialized set of physiological capabilities. Barneah *et al.* (2004) also indicated that hosts harboring either clade A or clade C symbionts co-occur in the same habitats. For example, *Litophyton arboreum* (clade A) and *Rhytisma fulvum* (clade C) form monospecific carpets on Eilat's reef flats (Benayahu & Loya, 1977). Furthermore, molecular analysis of zooxanthellae from *Heteroxenia, Sinularia, Rhytisma, Stereonephtya* and *Litophyton,* sampled over a depth gradient (1 to 20 m), reveals persistence include specificity with depth within a host (Barneah *et al.*, 2004). Therefore, the distribution of symbiont clades in Eilat's soft corals negates the correlation between symbiont clade and depth demonstrated in Caribbean reefs (Rowan & Knowlton, 1995; Rowan *et al.*, 1997; Toller *et al.*, 2001).

Whilst these studies are important to show emerging patterns (and one would presumably expect similar patterns to persist in closely connected Red Sea systems, such as those of Egypt's Red Sea), no study has yet examined sub-cladal identities, which is likely to be the actual determinant of how *Symbiodinium* genetic diversity may ultimately contribute to thermal stress sensitivity.

1.8. Approaches to monitor and predict coral bleaching

1.8.1. Observing bleaching

Over the past 30 years, coral bleaching has become a widespread phenomenon and is now seen by many as one of the most distinct manifestations of climate change impacts on natural ecosystems. Quantifying the scale of such events presents particular challenges. *In situ* underwater observation is clearly of limited utility due to the magnitude of observation required; for example, few reefs are within easy reach of research institutions or commercial dive centers. Furthermore, detecting bleaching, when it occurs, is not always as easy as it may appear, and is complicated by numerous physiological and physical factors that confound simple observations due to problems at both the individual colony scale (assessing whether a coral is bleached or not) and at the reef and regional scales (how large an area is bleached) (Baker *et al.*, 2008). Almost all bleaching monitoring is based on *in situ* observations by scientists, ecosystem managers, and trained volunteers. Standardized reef monitoring techniques, such as photo-quadrates and line point intercept approaches (e.g. English *et al.*, 1997; Hill & Wilkinson, 2004) provide the basis for most assessments of how much coral has bleached. Even when 'citizen science' type approaches are employed (such as Coral Watch), there is some concern as to the standardization with which bleaching is recognized and characterized (Suggett & Smith, 2011).

Bleaching can be described by matching affected colonies to a color scale to differentiate various degrees of paling, and thus by nature is a bio-optical phenomenon. Ranked scales of paling have been proposed (Gleason, 1993; Edmunds *et al.*, 2003; McClanahan *et al.*, 2004) that have eventually developed into more refined scales using a color reference card (Fabricius, 2006; Siebeck *et al.*, 2006). Some caveats apply to visual identification, especially by non-experts, since extreme polyp retraction (Brown *et al.*, 1994),

as well as disease and grazing, can be misinterpreted as bleaching. In addition, loss of algal symbionts begins long before bleaching becomes visually apparent (Fitt *et al.*, 2000). In some cases, chlorophyll-a level can remain unchanged despite significant changes in other pigments, such as peridinin, which respond to light and nutrients (Iglesias-Prieto & Trench, 1997).

Nevertheless, semi-quantitative data provided by color scales are generally considered useful for a synoptic description of bleaching status and have proved useful in rapid field surveys using towed observers, or downward-facing video cameras (Berkelmans & Oliver, 1999; Jordan & Samways, 2001; Kenyon *et al.*, 2006). Baird & Marshall (2002) used a simple categorization of degree of colony bleaching as follows: no bleaching, 1–10% bleached, 11–50%,51–99%, 100% bleached, dead; and placed all pale (but still partially colored) colonies in the 1–10% bleached category.

More quantitative bio-optical methods of evaluating coral bleaching have become available with satellite or remote sensing and aircraft based imaging sensors of sufficiently high resolution (Holden & LeDrew, 1998) across large reef areas or via kite photography (Hasegawa *et al.*, 1999), and aerial photography (Andréfouët & Riegl, 2004) at relatively small spatial scales. The loss in pigmented zooxanthellae from corals during bleaching events results in an optical signal that is strong enough for remote sensing to detect; however, this monitoring must still be linked to direct determinations of coral bleaching" on the ground" (i.e.*in situ*) (Holden & LeDrew, 1998; Myers *et al.*, 1999).

1.8.2. Bleaching 'products' for assessing future bleaching episodes

Contemporary approaches to "managing" bleaching now involve, either implicitly or explicitly, a variety of strategies to minimize bleaching risk or impact (Salm & Coles, 2001); two key types of strategy exist: (I) forecast the extent of anomalous environmental stress, i.e.

identify areas most potentially susceptible to bleaching. Typically, this approach uses coupled ocean-atmosphere climate models to forecast bleaching stress on reefs (Hoegh-Guldberg, 1999b; Donner *et al.*, 2005); or (II) estimate bleaching susceptibility for key taxa and predict how the biological communities will respond (McClanahan *et al.*, 2007a; Kleypas *et al.*, 2008), including attempts to use the relative abundance of heat tolerant *Symbiodinium* in corals to help identify ‘‘Reefs of Hope’’ that might be less bleaching susceptible (Baker, 2003; Baker *et al.*, 2004). Accurate coral bleaching forecasts can aid managers of marine protected areas to decide where to focus reef management efforts (Marshall & Schuttenberg, 2006; McClanahan *et al.*, 2007b). Also, accurate forecasts are necessary to assess what levels of greenhouse gases prevent dangerous, perhaps irreversible, climate change impacts for corals (Reaser *et al.*, 2000) and what levels allow coral reef ecosystems to adapt to climate change, as required by the second article of the United Nations Framework Convention on Climate Change (Oppenheimer & Petsonk, 2005).

The most important question in bleaching forecasting (currently) is: how much heat (or light etc.) is needed (anomaly excursion, length, timing) to cause bleaching? To date, this question has really only been tackled from the point of view of temperature given that it is relatively easy to measure by remote sensing but also that it is the primary factor associated with mass bleaching (even if moderated by other factors such as light, flow etc.). The heat threshold idea was pioneered by Glynn (1993, 1996), Goreau & Hayes, (1994, 2008) and Hoegh-Guldberg (1999b), and further by Fitt *et al* (2001) and Berkelmans (2002). The original idea was that upper and lower temperature thresholds exist which, when exceeded, result in physiological stress resulting in the breakdown of symbiosis. Exactly how these thresholds are defined, whether they need to be exceeded only once, or repeatedly for how

long, and by how much, as well as the role of past temperature variability, is still debated today(Manzello *et al.*, 2007; McClanahan *et al.*, 2007b).

Indicators used to forecast bleaching episodes have included monthly mean sea temperatures above a local threshold (Goreau & Hayes, 1994; Brown *et al.*, 1996) as well as cumulative heat stress (Gleeson & Strong, 1995; Podestá & Glynn, 1997) and can be summarized in table 1-2.

Table **1-2**. Summary of different prediction indexes of corals bleaching using regional anomalies of water temperature that always acquired from remote sensing

Index	**Degree Heating Week (DHW)**	**Degree Heating Month (DHM)**
Definition	Measurement of accumulated thermal stress over a 12-week period above the highest summertime mean. It is combining the intensity and duration of thermal stress into one single number	The cumulative sum of anomalies more than 1ºC above monthly averages, and used this index to identify ocean ‘‘hotspots’’
Calculation	One DHW is equivalent to one week of sea surface temperatures. The unit for DHW is "°C-weeks".	HotSpot = SST (°C) above the maximum monthly mean (MMM). The values of HotSpot show the number of degrees Celsius above the maximum monthly mean.
Advantage	Coral bleaching prediction needs some way to not only measure how far the temperature is above the threshold, but also how long it has stayed above.	HotSpot maps highlight those areas around the world that corals start to become stressed where sea surface temperatures are above the maximum monthly mean (MMM).
Usage	DHWs have been fairly successful in predicting coral bleaching events, and have been incorporated into NOAA’s *Coral Reef Watch program*. (Strong *et al.*, 2006)	Donner *et al.* (2005b) stated that bleaching was projected for all global reef locations by calculating degree heating months (DHMs) and reported that biennial bleaching is projected for 95–98% of all reefs by 2050–2059 in the A2 scenario.
Reference	(Liu *et al.*, 2006)	(Goreau & Hayes, 1994)

McClanahan *et al.* (2007b) observed that DHWs, combined with information on past temperature anomalies and coral community sensitivity, only predicted about one-half of Indian Ocean bleaching in 2005, suggesting that these metrics might not be good predictors of milder bleaching events. However, the NOAA coral bleaching monitoring program that uses a 5-km-resolution data set was not registering thermal anomalies signaling a major bleaching event, although conditions conducive to bleaching were indicated (Strong *et al.*,

2006). Thus, the bleaching indicators currently in use may not be applicable/appropriate. In part this may reflect the need for more regional parameterization of the nature and extent of the bleaching process to thermal anomalies (and importantly the extent of temperature anomaly that results in bleaching).

1.9. Towards better predicting coral bleaching in Egypt's Red Sea (thesis aims)

According to van Hooidonk & Huber (2012), the problem of predicting thermally induced coral reef bleaching can be divided into discrete components; I) having an accurate climatology and a thermal threshold above which bleaching is projected to occur; II) long-term (century) trends in annual mean temperatures; III) predicting the changes in temperature seasonality on long-time scales (the long-term trend in seasonality); (VI) predicting the spatial pattern of these trends; (V) predicting the inter-annual, decadal, and multi-decadal variability around these trends. Rising to this challenge requires that we scale key important findings and factors to larger regions (Langmead & Sheppard, 2004; Wooldridge & Done, 2004), test regional environmental models with field data, and improve the understanding of causation between environmental histories, acclimation/adaptation, and survival in order to evaluate and predict the future of climate on corals (Wooldridge *et al.*, 2005).

Currently it is well known that high SSTs above some site-specific threshold will lead to coral bleaching and mortality but that this threshold is sensitive to the taxa, regions, and their associated environmental backgrounds (Coles *et al.*, 1976; Coles & Brown, 2003; McClanahan, 2004); not surprisingly a targeted regional approach must, therefore, be taken in order to develop meaningful long-term bleaching management tools for those specific regions in question. Unfortunately, to date almost all data for the Red Sea has come from a single site (Eilat ~ 10 km long) and very little is known about the nature and extent with

which the Red Sea coral communities, and specifically those associated with Egyptian waters, are and will be susceptible to thermally induced bleaching.

Due to thermal tolerance/susceptibly of corals can be influenced by several environmental, physiological and genetic factors as summarized in Figure (1-5). This thesis, therefore, aims to better understand the susceptibility of Red Sea coral communities to

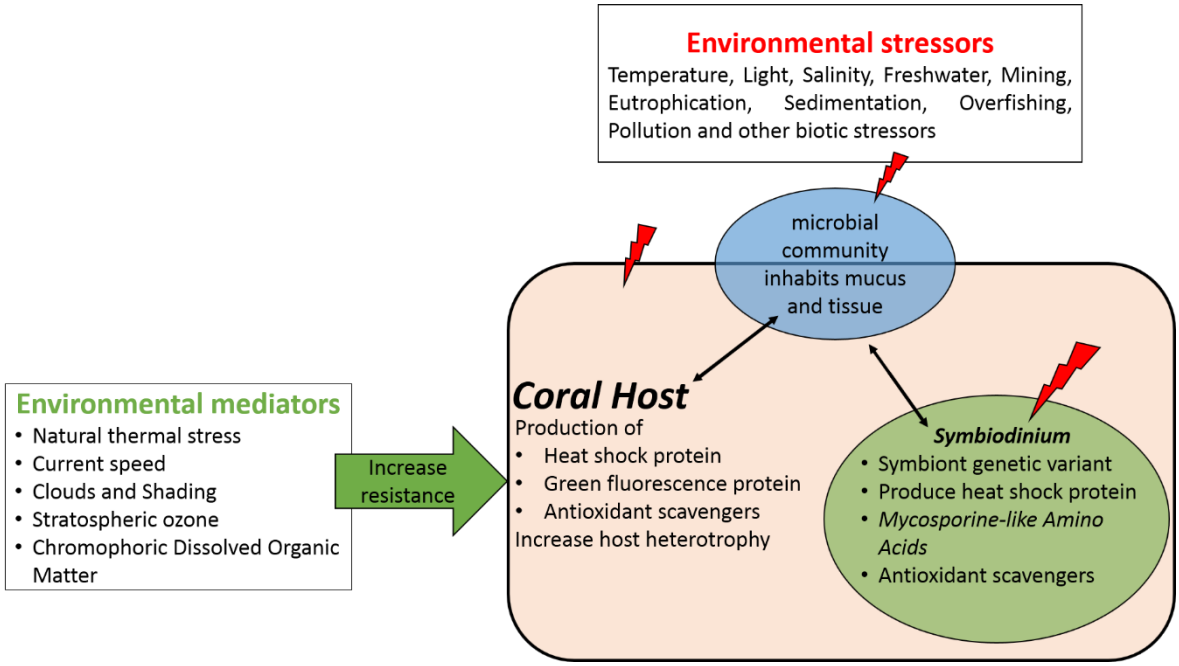

Figure **1-5.** Conceptual model demonstrates underlying mechanisms of thermal tolerance of reef-building corals. The environmental stressors (e.g. light, temperature, etc) influence each component of the holobiont (e.g. host, *Symbiodinium* and microbial community) which resist the stress using different mechanisms. **I**) Physiological flexibility of coral host determines its tolerance; specifically, host can produce chaperon protein (e.g. heat shock protein) to repair damaged proteins, green fluorescent protein as photo-protection, and produce ROS scavenger to minimize its toxicity effect. Heterotrophy capability and lipid reserve of host can be determining factor to tolerate the anomalous events. **II**) Symbiont genetic variant is another determining factor influence coral tolerance where tolerant clades (e.g. clade D) have high physiological flexibility and the potential to produce ROS scavenger and heat shock protein as well as photo-protective Mycosporine-like Amino acid. **III**) However microbial community is influenced by environmental variables, it can also be influence by availability of nitrogen wastes of host/*Symbiodinium* (e.g. ammonia, nitrate, etc). The microbial community change can be either negatively or beneficially affect the coral host and zooxanthellae. Bacteria can provide various functions include, but not restricted, nitrogen and carbon fixation and can provide supplementary food source for the host during the stress event. Also microbial community can produce antimicrobial substances, ROS scavenger or heat resistant protein which fill specific environmental niche. On the other hand, **IV**) some environmental variables can mediate the effect of stressors by removing ROS through high speed of water flow or reduce light/UV stress via Chromophoric Dissolved Organic Matter (CDOM) and stratospheric ozone. Also, corals inhabit naturally high thermal regime can provide tolerance either through phenotypic plasticity or natural selection of tolerant genotypes (e.g. *Symbiodinium* spp and microbial community). This model explains that total microbiome (i.e. *Symbiodinium* and microbial community) can play a crucial role in thermal susceptibility in corals inhabit naturally high sea surface temperature.

thermal anomaly induced bleaching by (I) Evaluating the existing data of Red Sea corals bleaching mortality and its relationship to taxa and environmental conditions/history; (II) Determine the anomalous temperature conditions (thresholds, duration) required for bleaching-induced mortality between key species and environments. (III) Identify the relative role of the microbiome (symbiont genetic variant vs microbial community composition) associated with corals at different thermal regimes to drive the corals' acclimation.

The hypothesis of this thesis is that the northern Red Sea will be more susceptible than central/southern area and require less thermal anomalies to drive severe coral bleaching. Also, corals in the Red Sea may harbor thermal tolerant clades and microbial community that increase its thermal tolerance in comparison to elsewhere. Consequently, the thesis structure will be as the following:

1. Chapter 2: characterize the spatial and temporal variations of environmental variables across the Red Sea and how they influenced past bleaching episodes. The objectives for this chapter is to investigate the historical pattern of environmental variables induce bleaching and how they driven the historical bleaching episodes. The hypothesis of this chapter propose that the northern Red Sea is more susceptible than central-southern Red Sea to, particularly, SST anomalies which caused, for the first time, massive bleaching pattern in the northern Red Sea. The approach is analyzing remote sensing data during last three decades and investigates its correlation to mass bleaching episodes collected from old bleaching surveys and published reports across the Red Sea.
2. Chapter 3: investigates the composition and reorganization of the microbiome communities across different latitudes experiencing different thermal history in the northern Red Sea. The hypothesis of this chapter is that both *Symbiodinium* and microbial community will significantly change across latitudes as a response to increase of water

temperature southwards. Hence, the objectives are to investigate the structure of the microbiome associated with coral species inhabit across latitudes and, therefore, the approach is to identify the microbial community and symbiont genetic variant (i.e. zooxanthellae) associated with six key coral species exist across the Egyptian Red Sea coast.

3. Chapter 4: examines experimentally the potential tolerance of corals inhabiting different thermal regimes (northern-Hurghada vs central-Jeddah) and the role of microbiome in the acclimation/adaptation process. This chapter investigates the assumption there is a regional difference in thermal susceptibility of reef-building corals across the Red Sea, and thus corals will be influenced to different extend to thermal anomalies via change microbiome community composition. Therefore, the objective is to experimentally investigate the physiological status of corals inhabiting different regions after being exposed to thermal stress (+3˚C over local summer mean) and how the microbiome community will change in a response to heat stress. The hypothesis here is that northern will be more susceptible than the central Red Sea and will face decline in its physiological state. Also, it is proposed that the central Red Sea harbors more tolerant symbiont variants than northern Red Sea which induce reef-building corals to be more thermally tolerant.

Chapter 2

2. *Historical environmental variables induced corals bleaching in the Red Sea*

2.1. Abstract

Coral reefs inhabiting environmentally extreme habitats provide clear evidence as to how corals can adapt to stressors and potentially future climate change. The Red Sea is a "black box" and little is known about the inherent variability of key environmental variables (e.g. temperature, chlorophyll-*a*, light attenuation) that influence coral fitness or how this variability ultimately drives sensitivity to thermally induced mortality. Work presented here addresses this knowledge gap and examines remotely sensed Sea Surface Temperature (SST), Chlorophyll-*a* (Chl-*a*) and light attenuation (K_d) to reveal spatial differences along the Red Sea. Overall, the Red Sea was warmer, more productive and turbid in its southern region ($p<0.001$), however, it experiences lowest seasonal SST range. Differences in the environmental profile of the Red Sea was driven mainly by SST (PCA-88%) although Chl-*a* was also important especially in the southern region (PCA-11%). The characteristics of thermal anomalies/stress have changed dramatically since 1995 and intensively post 2007, particularly at the northern Red Sea. This trend was associated with bleaching at the central Red Sea, while extreme tolerance at far north Red Sea and Gulf of Aqaba (10.9 and 15.1°C-weeks in 2010 at Hurghada and Gulf of Aqaba respectively). Analysis of vulnerable coral taxa from past bleaching episodes indicated that species of *Porites, Pocillopora, Acropora, Stylophora* and *Millepora* were the most frequently bleached genera. Surprisingly, and in contrast to many other bioregions, massive coral species were observed to bleach more frequently (45.6%) than branching species (36.3%). The results show for the first time, through mining of previously published, unpublished data sets and remote sensing data, that the central/central Red Sea will be more influenced by thermal stress, while corals in northern

Red Sea and Gulf of Aqaba experienced extreme thermal tolerance. This raising the importance of constructing regionally specific thermal stress models to investigate spatial variation in coral susceptibility and whether these regions dominated by tolerant corals could be considered as potential corals refuge.

2.2. Introduction

The relationship between the coral host and its symbiotic algae species (genus; *Symbiodinium*) is optimized to a relatively narrow window of environmental conditions (e.g. light and temperature) (Drew, 1972; Hoegh-Guldberg & Smith, 1989), and small atypical "shifts" (or anomalies) of one or more of these variables can disrupt the viability of this symbiotic relationship (Glynn, 1990). Coral bleaching is a conspicuous response to Sea Surface Temperature (SST) anomalies, (Hoegh-Guldberg *et al.*, 2007; Veron, 2008), but can also result from changes in light, salinity and nutrient availability (e.g. Baker *et al*., 2008; Suggett & Smith, 2011). However, thermal thresholds often vary by region, e.g. upper thermal thresholds can be as low as 27^{o}C at Rapa Nui in the South Pacific Ocean but as high as 35 °C in the Persian Gulf (Wellington *et al.*, 2001; Coles & Brown, 2003; Riegl, 2003) and anomalous SSTs as little as 1°C above the long-term average can trigger bleaching (e.g. Glynn, 1991; Goreau & Hayes, 1994; Hoegh-Guldberg, 1999; Weeks *et al.*, 2008). The actual extent of SST perturbation required to induce bleaching is not constant over space and time, reflecting regional and/or localized acclimatization to different thermal histories (Coles & Brown, 2003; Suggett & Smith, 2011), and is heavily dependent on the duration (e.g. Anthony *et al.*, 2007) and periodicity (Pratchett *et al.*, 2013) of the anomaly.

Red Sea coral reefs span 17.5° of latitude (12.5-30^{o} N) and grow in one of the warmest and most saline (average ~41 ‰) seas in the world (Behairy *et al.*, 1992; Fouda *et al.*, 1994). However thermal regimes are highly variable and within the central Red Sea, where SST

exhibits a seasonal range of up to 10 ^{o}C and summer temperatures often exceed 32^{o}C (Davis *et al.*, 2011) corals do not often appear to bleach even when temperatures reach upwards of 34°C (Grimsditch & Salm, 2006). As such, Red Sea corals have already been proposed to exist in "future thermal environments" that are predicted for most tropical reefs in the future (Grimsditch & Salm, 2006). Coral species of the Red Sea thus appear highly thermo-tolerant and have been predicted to be amongst the last reefs that will bleach as global SSTs increase (Grimsditch & Salm, 2006). Consequently, the more northerly reefs of the Red Sea have been considered as a potential "climatic refuge" for corals species (Gulf of Aqaba; Fine *et al.*, 2013). However, recent field observations have reported mass-bleaching episodes from the northern/central Red Sea (HEPCA, 2012; Furby *et al.*, 2013) raising the question, are environmental conditions that induce bleaching beginning to exceed the high thermal thresholds of the northern Red Sea corals?

Similar to the Red Sea, corals of the Arabian Sea exhibit the highest thermal threshold ever recorded (36 °C) (Coles, 2003) temperatures that would result in bleaching and mortality of most other species and region (Hume *et al.*, 2013). One theory is that the coral symbiont, *Symbiodinium*, may play an important role in the coral's tolerance to such high thermal conditions. Research was carried out by Hume *et al* (2015) potentially discovered a new species of zooxanthellae (*Symbiodinium thermophilum)* which may enable corals of the Arabian Gulf to tolerate thermal stress (and potentially high salinity) known to cause mass mortality elsewhere.

In the Red Sea, large environmental gradients that run north-to-south, have recently been documented to induce changes to the coral acclimation (Sawall *et al.*, 2014). However, bleaching prevalence and the key environmental factors driving susceptibly, remains largely unknown for the Red Sea and few published papers have considered bleaching in this

bioregion (see; Berumen *et al.*, 2013). Such a general lack of knowledge precludes establishment of a historical "baseline" with which to evaluate current and future changes in environmental conditions and coral stress responses. As a first step to addressing these unknowns, the historical variability in key environmental variables that have previously been linked to bleaching (temperature, turbidity, and chlorophyll-*a*) was investigated, and compare this environmental variability to bleaching events on a local and regional scale.

In doing so, the research provides the first detailed characterization of the spatial and temporal variation of key environmental variables throughout the Red Sea in association with regionally bleaching events. The objective is to investigate the remote sensing data over past three decades and how it driven the past bleaching episodes which obtained from publications and bleaching survey. The hypothesis of this work is that the northern Red Sea is more susceptible to thermal stress and require less SST anomalies to drive massive bleaching which explain recent observed bleaching in central and part of the northern Red Sea.

2.3. Materials and Methods

2.3.1. Study Sites

The Red Sea is a unique basin of semi-enclosed water body boarded by eight countries that spans between 30^{o}N and 12^{o}30′ N, of ca. 2000 km of navigable water. The Red Sea is linked to the Indian Ocean in the south through the Gulf of Aden via the Strait of Bab El-Mandeb and to the Mediterranean Sea in the north via the Suez Canal. Along the Red Sea coral reef bioregion, six sites were targeted (see Fig. 2-1 and Table 2-1) to represent (i) different latitudes along the Red Sea coast as well as (ii) past coral reefs bleaching observations. Sites were Gulf of Aqaba and Gulf of Suez to represent both associated Gulfs connected to the proper body of the Red Sea. Hurghada represents the northern Red Sea,

while Wadi El Gemal and Jeddah represented central parts of the Red Sea, and Farasan Island located on the Saudi coast, represents the southern Red Sea (see. Table 2-1)

Table **2-1**. Coordinate of study sites across the Red Sea where remote sensing data were acquired (grid of 4 km^2) to investigate the historical environmental variables.

Site	location	Lat.	Lon.
Aqaba	Abo Ghaloum	28.658	34.604
Suez	Zafarana	28.955	32.668
Hurghada	Fanous	27.239	33.862
Wadi El Gemal	Sharm El Fukary	24.698	35.132
Jeddah	Thuwal	22.234	38.979
Farasan	Farasan	16.227	42.439

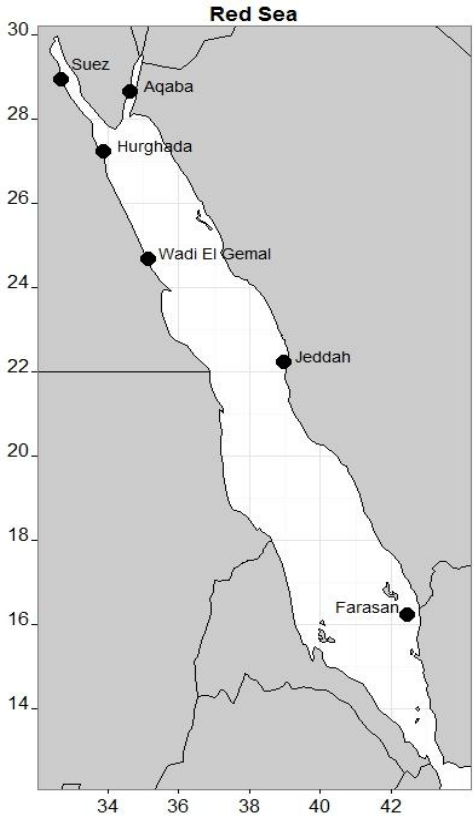

Figure **2-1**. Map of the study area show Red Sea and borders countries as well as study sites (black dots) along different latitude of the Red Sea to study the environmental variables history across Red Sea.

2.3.2. Environmental variables

Three key environmental variables known to induce coral bleaching were examined along the Red Sea. (i) Sea Surface Temperature (SST) (Donner *et al.*, 2005; Hoegh-Guldberg *et al.*, 2007), (ii) Chlorophyll-*a* (Chl-*a*) as indicator of water productivity (Maina *et al.*, 2008; Fabricius *et al.*, 2013), and (iii) Diffusion Attenuation Coefficient ($K_{d\ 490m}$), which describes the extent of light penetration/turbidity (Suggett *et al.*, 2012). Weekly Sea Surface Temperature (SST) records were acquired from the Coral Reef Temperature Anomaly Database (CoRTAD) from Pathfinder 5.2 (4 km resolution) (http://data.nodc.noaa.gov/cortad/), which has been used previously to quantify thermal stress patterns of coral reefs in other bioregions (see; Liu *et al*. 2006; Eakin *et al*. 2010). Weekly Sea Surface Temperature (SST), weekly Sea Surface Temperature Anomalies (SSTA), and weekly Thermal Stress Anomalies - Degree Heating Weeks (TSA-DHW) datasets were extracted from study sites coordinates for the period

between and including 1981 to 2012 (CoRTAD, Version 5) using ArcGIS 10.3.1 software. SST metrics were used and derived from CoRTAD remote dataset for each study site to quantify historical thermal stress along the Red Sea, and it can be described and calculated as the following;

1) Sea Surface Temperature Anomalies (SSTA) is the positive/negative values of SST that exceed long term mean SST for the study site (equation 2-2). This indicates that current SST is warmer/colder than usual. SSTA is calculated by subtracting weekly SST from weekly climatology (long-term mean for each week/month, see equation 2-1) as described below;

$$Climatology = \frac{\sum_{2012}^{1982} weekly\ SST}{n} \quad \text{[Equation 2-2]}$$

$$SSTA = weeklySST - weekly\ Climatology \quad \text{[Equation 2-1]}$$

2) Thermal Stress Anomaly (TSA), known also as "HotSpot" (Strong *et al.*, 1997), is the SST when exceed maximum summer long-term mean (maximum Climatology). TSA quantify the occurrence and magnitude of positive SST anomalies by excessing the regional SST above the maximum summer long-term mean. TSA or HotSpot can be calculated as in equation 2-3;

$$TSA\ (HotSpot) = Weekly\ SST - max.weekly\ Climatology \quad \text{[Equation 2-3]}$$

3) Bleaching Threshold is the SST after exceeding maximum summer long-term mean by 1 °C which is sufficient temperature to induce coral bleaching (Glynn & D'Croz, 1990). Bleaching thermal Threshold is calculated as in equation 2-4;

$$Bleaching\ Threshold = Max.weekly\ Climatology + 1\ ℃ \quad \text{[Equation 2-4]}$$

4) Degree Heating Weeks (DHWs), known also as TSA-DHW, is an accumulative index of thermal stress over time site (Liu *et al.*, 2006) which is take the prolonged duration

of thermal stress into account. DHW is sum of previous 12 weeks when thermal stress anomalies (TSA or HotSpot) is ≥1 °C, and it is calculated as in equation 2-5;

$$DHWs = \sum HotSpot \geq 1\,℃\ for\ previous\ 12\ weeks \quad \text{[Equation 2-5]}$$

Monthly Chlorophyll-a content (Chl-*a*, mg/m^3) and Light Attenuation specific to 490nm (K_d, m^{-1}) were also derived for each study site for the period 2003-2012 using Giovanni Ocean Colour tool; (http://giovanni.gsfc.nasa.gov/giovanni/), from the Moderate Resolution Imaging Spectro radiometer (MODIS) Aqua satellite (4 km resolution). For Chl-*a* and K_d variables, values for each month (m) were averaged across all years to yield the Climatology (X_m) [equation 2-6], or monthly long term mean (± standard deviation), to subsequently calculate the monthly anomaly (A_m) and annual anomalies (A_a) as positive and negative deviations from their climatology (monthly long term mean) according to Maina (2008) as in equations 2-7 and 2-8.

$$X_m = \frac{\sum_{2012}^{2003} m}{ny} \quad \text{[Equation 2-6]}$$

Xm= average month value (*Jan 2003, Jan 2004,*…etc.), *ny*= number of studied years (10 years for Chl-*a* & K_d)

$$A_m = (X_m - Y_m) \quad \text{[Equation 2-7]}$$

Ym= monthly value for each year (Jan 2003, Feb 2004,…etc.)

$$A_a = \frac{\sum_{Dec}^{Jan} A_m}{nm} \quad \text{[Equation 2-8]}$$

nm= number of months (12 months).

2.3.3. Bleaching Incidences

Past bleaching data were collated to evaluate the extent of bleaching during 1982-2012 as well as the most frequently bleached coral taxa throughout the entire Red Sea. Data were obtained from the following sources;

1-Reef base database (http://www.reefbase.org/main.aspx) of bleaching severity, data deposited by both researchers and citizen scientists or acquired from published literatures. This dataset provided total bleaching percentage in 66 surveyed sites alongside the Red Sea (1998-2010).

2-Annual Reef Check survey conducted by volunteers at random locations along the Egyptian Red Sea coast from 1997 to 2012. Survey was undertaken based on point intercept line transect protocol (20 m, 0.5m interval) covering a total area of 400 m^2 of coral reef according to Reef Check standard protocols (Hodgson *et al.*, 2004).

3-Previously published papers/reports, unpublished sources (>20) and anecdotal data from personal communications with local researchers and divers (see Table 2-4). As the Red Sea had very few mass bleaching events (e.g. 1998, 2010, 2012), the bleaching data for each event collected as the following; 1) during 1998, data were collected by SCUBA swimming per time effort (30-40 min) counting all coral colony, and then total bleaching percentage was quantified (see Devantier & Pilcher, 2000; DeVantier *et al.*, 2000; Pilcher & Devantier, 2000; Pilcher & Nasr, 2000). For 2010 and 2012 bleaching events (see. HEPCA, 2012; Furby *et al.*, 2013), data were collected using line transact intercept method (as per English *et al.*, 1997; Hodgson *et al.*, 2004).

All bleaching data were normalized into bleaching percentage relative to total coral cover, then tabulated and bleaching severity was ranked according to Thompson and van Woesik (2009) as: (i) natural mortality (no bleaching) (<1 %); (ii) low bleaching (1–10% of

corals bleached); (iii) medium bleaching (10–30% of corals bleached); and (iv) high bleaching (>30% of corals bleached). To identify susceptible coral taxa during the bleaching events (i.e. 1998, 2007, 2010 and 2012), bleached genera was reported and their frequency were estimated according to presence/absence in mass event. Also, the growth form for each genus/species were assigned into four categories (i.e. massive, branching, soft and encrusting corals) as possible and total percentage for each growth form was calculated.

2.3.4. Data Analysis

One-way ANOVA were used to examine the differences between sites or years separately for all metrics of environmental history (i.e. annual mean of weekly SST, SST anomalies, Chl-*a*, K_d as well as annual Max. DHW) after normality test (Shapiro test) and log/square root transformation if needed. This followed by *Post hoc* Tukey to test specifically which site was significantly different ($p<0.05$). Annual mean of environmental variables (SST, Chl-*a* and K_d combined) for the period 2003-2012 were used to perform Principle Component Analysis (PCA) to identify the environmental variable that drive the variability among sites. Raster maps for different SST variables/metrics were plotted directly from CoRTAD-v5 file (NetCDF format) after being cropped for the Red Sea coordinates. SST mean, max and minimum values transformed from Kelvin (K) to Celsius (°C) degree (i.e. SST - 272.15). All statistics and figures were carried out using 'R' statistical software (R Developement Core Team, 2015).

2.4. Results

2.4.1. Across site variance of environmental histories

As expected, overall SST mean throughout the period 1982-2012 (n=1617) declined significantly (ANOVA, d5, 1874, $p<0.001$) with increasing latitude (i.e. northward) within range of the Red Sea sites (Fig. 2-2, Table 2-2), although the Gulf of Aqaba was significantly warmer (Tukey's test- $p<0.001$) than Gulf of Suez for the same latitudes (Fig. 2-2). The mean SST ranged from 29±2.1 °C in Farasan to 23.6±3.2 and 21.7±2.1 °C in Gulf of Aqaba and Suez respectively (Table 2-2, Fig. 2-3). SST max decreased northward; i.e. ca. 33.9 °C in Farasan to 28°C and 28.8 °C in Gulf of Suez and Aqaba respectively (Fig. 2-2, Table 2-2), but minimum SST contrast this pattern (15.4 °C and 18.5 °C in Gulf of Suez and Aqaba respectively to 23.5 °C in Farasan) (Fig. 2-2, Table 2-2). The seasonal variability of SST (i.e. winter to summer range) varied across sites without defined spatial pattern (Table 2-2, see also Figure S1- appendix).

Data analysis confirmed that the warmest year at the northern Red Sea was 2010 (i.e. annual SST mean was 28.8 °C in the Gulf of Aqaba, 28 °C in Gulf of Suez, 29.9 °C at Hurghada, and 31.6 °C in Wadi El Gemal), while the southern Red Sea (Jeddah and Farasan) experienced highest annual SST during 1998-1999 (33.7 °C and 33.9 °C respectively) during 1982-2012 (Fig. 2-3). However, the trends of SST annual mean showed increase overtime at all sites (regression, $p<0.001$), but general decline southward was observed (Fig. 2-3). Specifically, linear regressions of annual SST mean (°C) versus time (year, n=31) yielded regression coefficients (°C/year) that were higher for northern sites ($10\pm2\times10^{-5}$ °C/year at Aqaba [$r^2=0.65$], Hurghada [$r^{2=}63$] and Wadi El Gemal [$r^2=65$]) than southern sites (Farasan, $6\pm2\times10^{-5}$ °C /year - $r^2=0.2$) (Fig. 2-3).

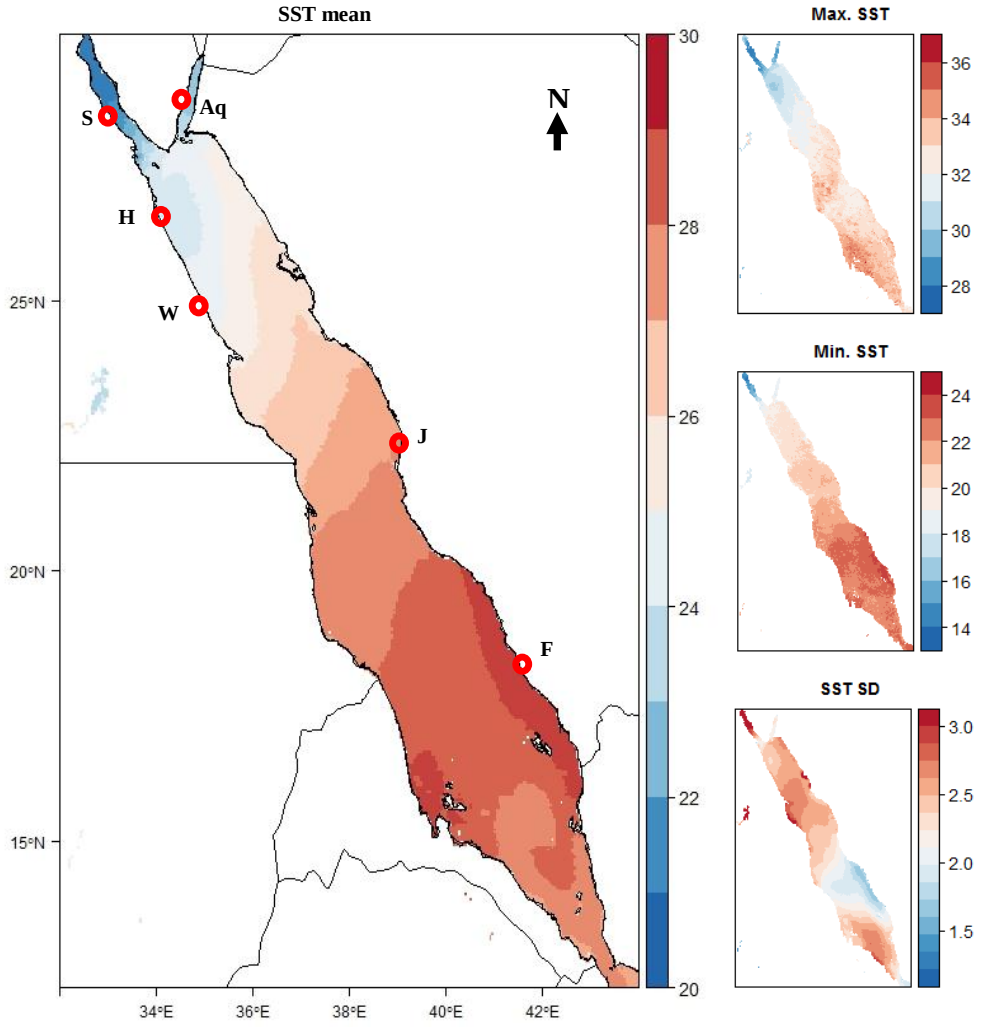

Figure **2-2**. Remote sensing maps demonstrate spatial distribution of mean, maximum, minimum and standard deviation (SD) of SST across the Red Sea (sites indicated by red circle and first letter of site name) during last three decades (1982-2012). Data obtained from Pathfinder 5.2-AVHAR-4km resolution (available online). The maps manipulated and clipped directly from CoRTAD-V5 global SST variables using "raster" package in "R" statistical software. Maps show clear decline of SST northward with obvious SST decline at Bab Al Mandab as a result of upwelling and water exchange with Gulf of Adan. In addition, it is clear that Gulf of Aqaba is warmer Gulf of Suez, however SST mean at Gulf of Aqaba was similar to far north of Egyptian Red Sea coast. However maximum SST was geographically restricted to south and minimum SST was restricted to north Red Sea, but the highest temperature fluctuation, hence SST standard deviation, was showed at northern Red Sea and at south-west cost of the Red Sea near Eretria.

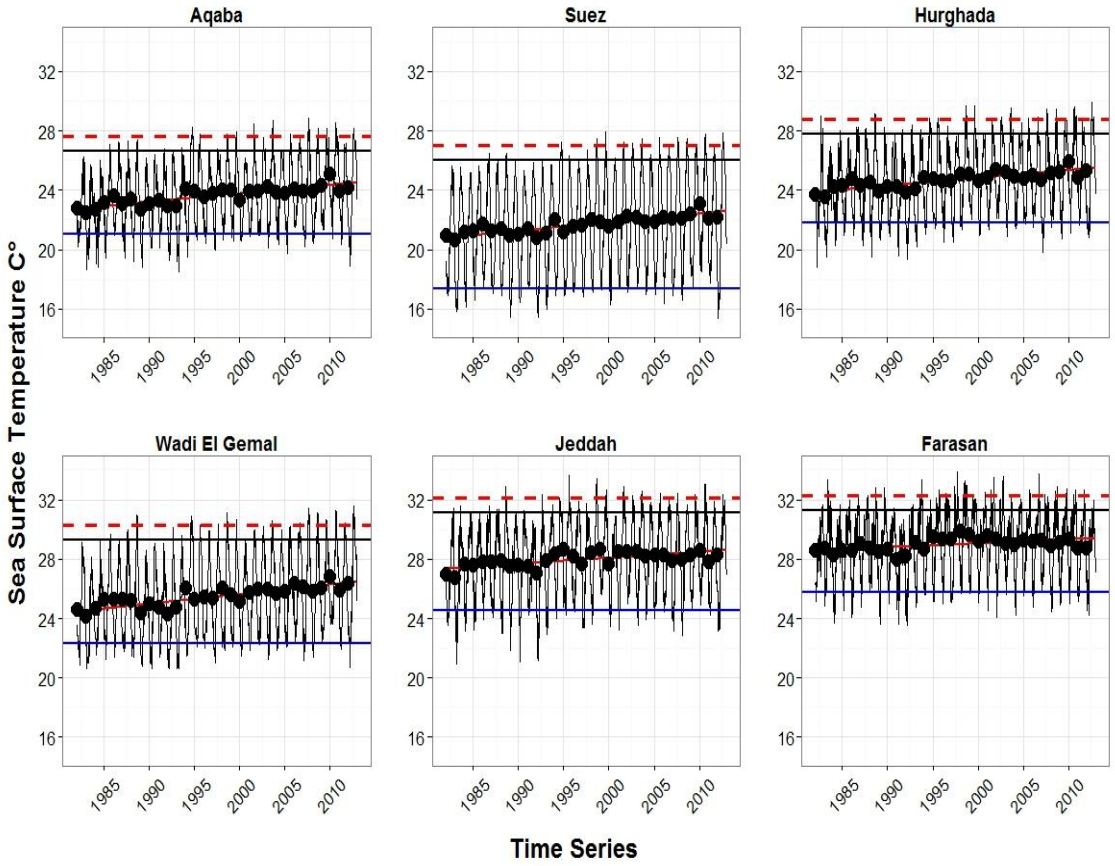

Figure **2-3**. Weekly time series (n=1617) plot of SST in six studied sites along gradient latitude of the Red Sea acquired from remote sensing data (CoRTAD - version 5, AVHRR Pathfinder 5.2, 4 km resolution) during last three decades (1982-2012). Each time series plot has four lines indicate; 1) Maximum summer mean (upper black solid line) and 2) Minimum winter mean (lower blue solid line) to show SST climatology/seasonal range for each site. 3) Bleaching thermal threshold (upper dotted red line) which is SST over maximum summer mean by 1°C as indices for bleaching, and 4) regression line (red solid line) indicate the trend of annual SST during last three decades. Symbols overlaid on time series demonstrate SST annual mean (n=31) in each site through the same period (1982-2010).

Table **2-2**. Summary of environmental variability ±SD (SST, Chlorophyll-*a* and $K_{d\ (490\ nm)}$) in different study sites along the Red Sea. Table shows the annual range (1982-2012) of SST, Climatology range, and seasonal variation (the difference between minimum and maximum climatology in each site), thermal threshold (SST higher than maximum monthly mean by 1 °C) as well as maximum and mean of Degree Heat Weeks (DHW).

Sites	**Suez**	**Aqaba**	**Hurghada**	**Wadi**	**Jeddah**	**Farasan**
Min. SST (°C)	15.4	18.5	18.9	20.6	21.0	23.5
Max. SST (°C)	28.0	28.8	29.9	31.6	33.7	33.9
Overall SST Mean (°C)	21.7±0.5	23.6±0.6	24.7±0.5	25.5±0.6	28.0±0.5	29.0±0.4
Min. Climatology (°C)	17.4±0.9	21±0.8	21.8±0.9	22.3±0.7	24.5±1.1	25.8±0.8
Max. Climatology (°C)	26.0±0.9	26.6±1	27.8±1	29.3±1.2	31.1±1	31.3±1.1
Seasonal range (°C)	8.6	5.6	6.0	7.1	6.5	5.5
Thermal threshold (°C)	27±0.9	27.6±1	28.8±1	30.3±1.2	32.1±1	32.3±1.1
Max. DHW (°C-week)	6.9	10.9	15.1	18.9	15.2	7.4
Mean DHW (°C-week)	0.3±1	0.6±1.8	0.7±1.95	0.9±2.5	0.5±1.7	0.5±1.3
Annual Chl-*a* mean ($mg.m^{-3}$)	0.35±0.03	0.19±0.06	0.23±0.03	0.36±0.06	0. 57±0.04	1.44 ±
Annual K_d mean (m^{-1})	0.05±0.003	0.04±0.01	0.04±0.003	0.05±0.004	0.074±0.003	0.074±0.0

Similar to the trend of SST annual mean, the trends of SST anomalies (i.e. SST deviation form weekly long term mean) exhibited a positive linear regression relationship over time ($p<0.001$ for all sites) (Fig. 2-4), and ranged from $10\pm2\times10^{-5}$ °C/year in Aqaba and Suez ($r^2=0.66$ and $r^2=0.67$ respectively) to $6\pm2\times10^{-6}$ °C/year ($r^2=0.2$) at Farasan. Overall, data confirmed that SST anomalies declined southward that contrasting the spatial distribution of SST mean that increased southward. Also, SST anomalies trend appeared to reflect an apparent long term shift from predominantly negative to positive anomalies from 1995 across all sites, indicative of a change to the overall heat stress for the region (Fig. 2-4).

Degree Heating Weeks (DHW) exhibited a similar shift consistent with SST anomalies, where DHWs were mainly below ca. 4°C–weeks during 1982-1995, while it showed predominant increase during last ca. 15 years particularly during periods of major El Niño-La Niña activity (i.e. 1998, 2010 & 2012) (Fig. 2-5). Spatial analysis of the maximum DHW within the period 1982-2012 confirmed this trend and further highlighted the migration from south to north throughout this time period (i.e. higher maximum DHWs for the southern Red Sea during the 1990s versus for the northern Red Sea in the 2000s) particularly, toward the southern Egyptian coast (Fig. 2-5A, see also Figure S2 - Appendix) which showed 15.1 and 18.9 °C-weeks during 2012 at Hurghada and Wadi El Gemal respectively (Fig. 2-5B). The linear regression trend of annual maximum DHW (n=31) was increased significantly (regression, $p>0.001$) overtime only in northern Red Sea (i.e. ranged from ca. $9\pm2\times10^{-4}$ °C–week/year at Wadi El Gemal [$r^2=0.38$] to $3\times10^{-5} \pm 9\times10^{-6}$ °C–week/year at Gulf of Suez [$r^2=0.33$]), while the trend was not significant overtime in southern Red Sea (Jeddah and Farasan, $p>0.05$) (Fig. 2-5B).

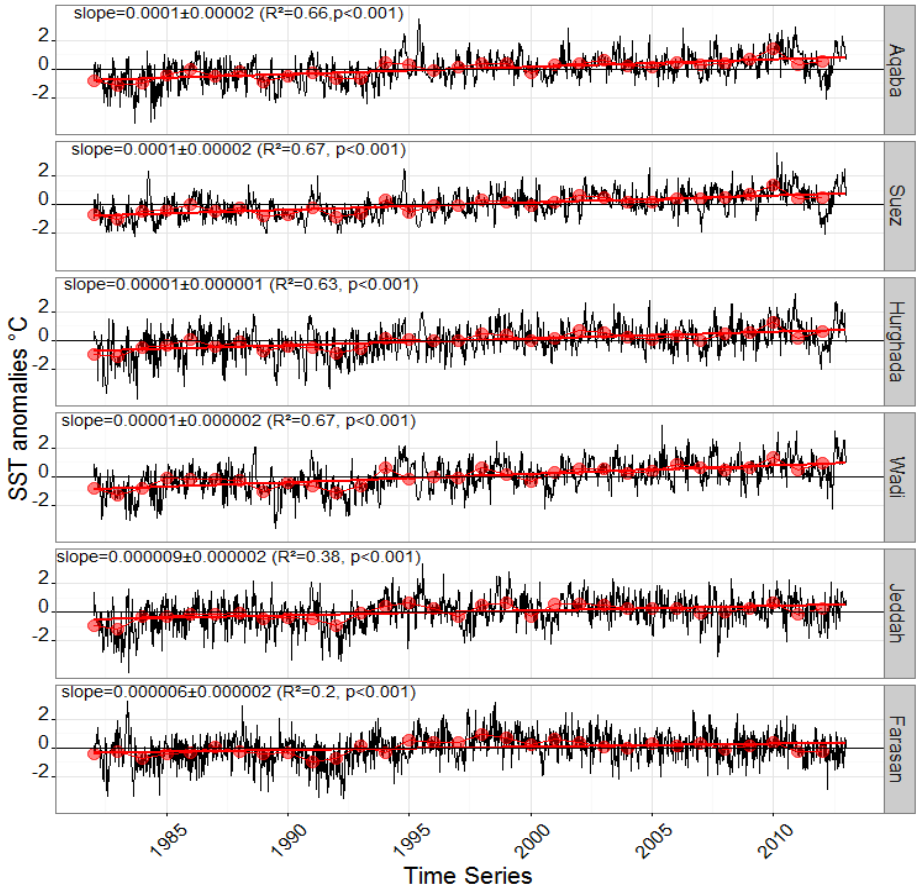

Figure **2-4**. Weekly Sea Surface Temperature Anomalies (SSTA) acquired from remote sensing data (CoRTAD -V5, AVHRR Pathfinder 5.2, 4 km resolution) during the study period (1982-2012) and calculated as differences between weekly SST and weekly climatology as in equation stated in material and methods. Ploy regression fitting line (red solid line) representing the best fit of SSTA for each study site.

To further assess the extent of DHWs and how they varied over space and time, all DHW data from 1982-2012 were subsequently placed into size bins according to field observation that showed; (i) <4°C-weeks (considered sub-lethal stress and generally not result any bleaching), (ii) significant bleaching >4 but <8°C-weeks; and (iii) widespread bleaching with expected mortality at >8°C-weeks (Liu *et al.*, 2006; Eakin *et al.*, 2010, see also Coral Reef Watch). Using this criteria, total number of DHW incidents increased southward (e.g. 13 incidents at Gulf of Suez to 21 incidents at Farasan) however, >50% of DHW incidents at northern Red Sea sites (i.e. Gulf of Aqaba, Hurghada, Wadi El Gemal) were in both categories ii and iii (Fig. 2-5C). This demonstrates that the frequency of heating

pressure has generally increased overtime southward, but with a pronounced increasing intensity only at the northern sites for the past three decades.

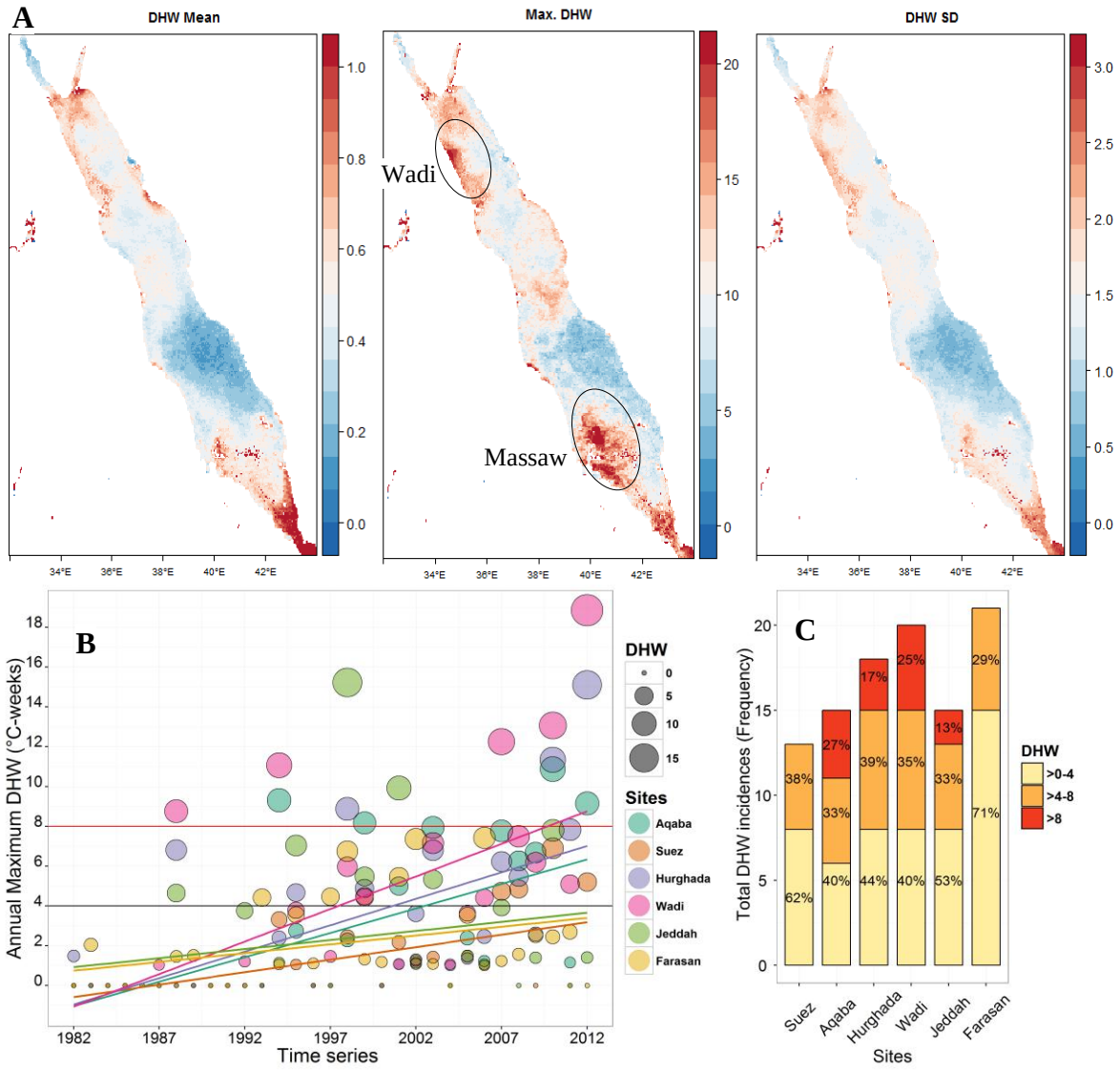

Figure **2-5**. Raster maps presenting DHW mean, max. and standard deviation (A) across the Red Sea through the study period (1982-2012). Data acquired from Pathfinder 5.2 (AVHAR-4km), CoRTAD-V5, and Red Sea clipped and manipulated from NetCDF file directly. Degree heating weeks (DHWs) at the study sites were plotted as maximum value per year in sized symbol scatter plot (B) to demonstrate the temporal variation of DHW. The reference line at 4 and 8 °C -weeks (B) represent worldwide field observations indicated that there is a correlation with coral bleaching when DHW values reached 4 °C-weeks and widespread bleaching and expected mortality when reach 8 °C-week. Accordingly, all bleaching incidents were categorized into three groups; > 0-4, >4-8 and >8 °C-weeks in each study site and plotted in the staked bar plot (C) for quantifying the effect of DHW on each site, and percentage calculated and overlaid for each category in each site.

Chlorophyll-*a* concentration (Chl-*a*) and the light attenuation coefficient (K_d) properties also differed across sites (Fig. 2-6). Mean values for Chl-*a* and K_d (2003–2012) were highest for the southernmost site (Farasan; 1.44 ± 0.26 mg.m^{-3} and 0.13 ± 0.02 m^{-1}) and generally declined northward and were lowest for the Gulf of Aqaba (0.19 ± 0.06 mg.m^{-3} and 0.04 ± 0.01 m^{-1}) (Fig. 2-6, Table 2-3). Thus, Farasan was not only the warmest, but also most productive and turbid site; in contrast, the northern most sites were the coolest and generally least productive/turbid. Whilst the Gulf of Aqaba exhibited a similar thermal regime as for Hurghada and Wadi El Gemal throughout this time frame, it exhibited clearer/less productive water (Fig. 2-6, Table 2-3).

Monthly Chl-*a* and $K_{d\,(490)}$ anomalies (deviation from long term mean as per equation 2-8) exhibited significant difference between sites (ANOVA, d5, 16.9, $p<0.001$), where Farasan exhibited a high fluctuation of Chl-*a* and K_d anomalies more than other sites (Tukey's, $p<0.001$), indicating that southern Red Sea is subject to relatively greater inter-annual variability of water productivity (see Figure S3 - Appendix).

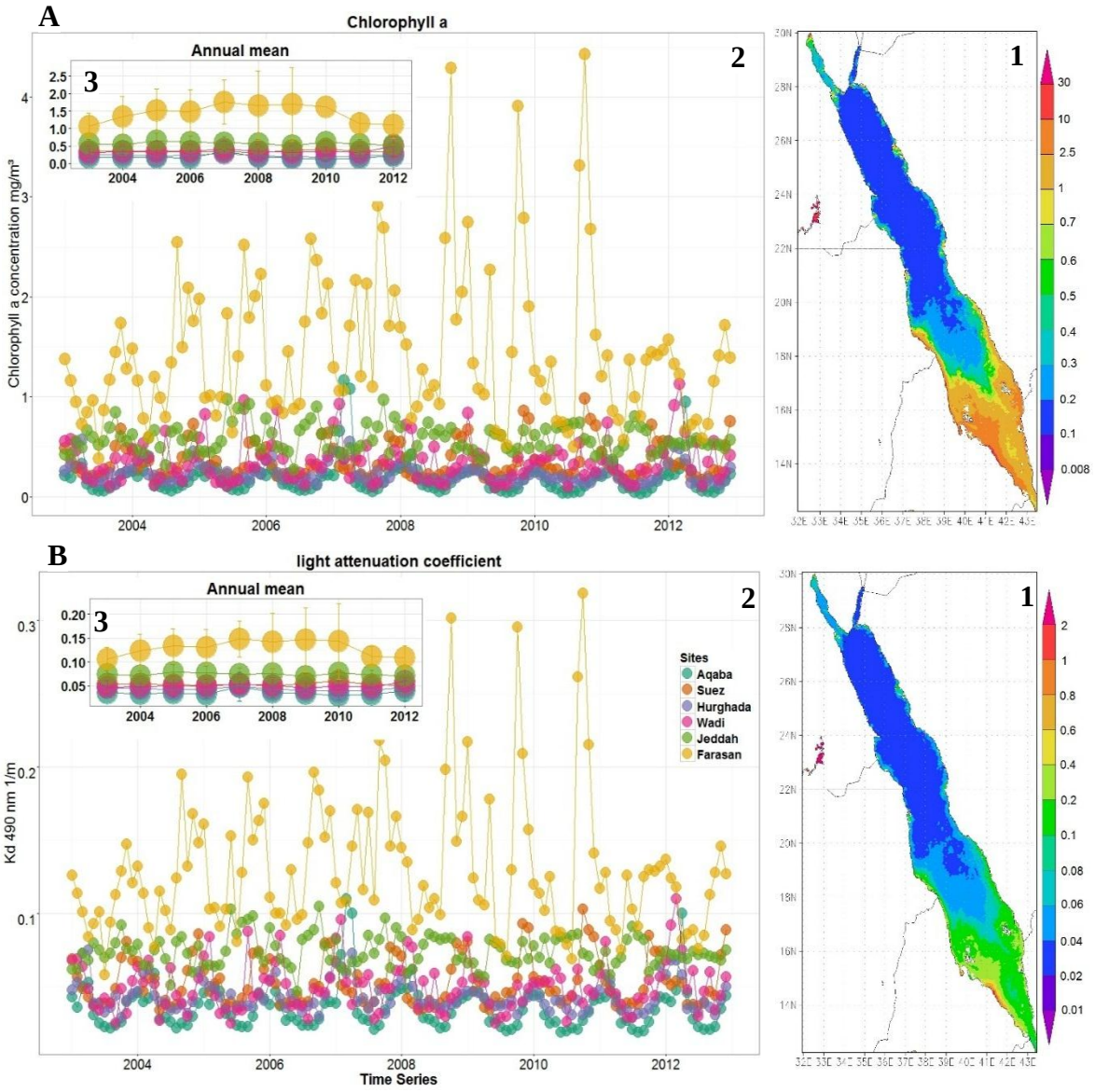

Figure **2-6**. Color coded maps (left) produced by Giovanni ocean color online tool during the period 2003 to 2012 and data obtained from remote sensing data (MODIS, AQUA satellite 4 km). Maps show high values of Chlorophyll-*a* concentration (A-1) and K_d 490 nm (B-1) in south Red Sea influenced by water exchange with Gulf of Adan and water upwelling. Monthly values (n=120) for Chl-*a* (A-2) and K_d (B-2) showed higher value in Farasan with high fluctuation values due to water exchange with Gulf of Adan in certain month in the year, hence high SD of annual mean (n=12) as shown in A.3 & B.3. Data demonstrate the capability of coral in southern Red Sea to live in high Chl-*a* & K_d range and fluctuations.

Finally, PCA analysis of Chl-*a*, K_d together (MODIS-Aqua) with SST (CoRTAD-V5) based on the annual means across the period from 2003-2012 (Fig. 2-7) demonstrated that SST was the major source of variation between sites (PC1, 88% of variation explained),

whereas Chl-*a* and K_d (PC2) together explained only 11.7% of variation (Fig. 2-7). From this, sites clustered as Gulf of Aqaba, Hurghada and Wadi El Gemal, and as Gulf of Suze (lower SST mean) and Jeddah (higher mean SST). In contrast, Farasan was less influenced by SST and more from Chl-*a* and K_d (Fig. 2-7).

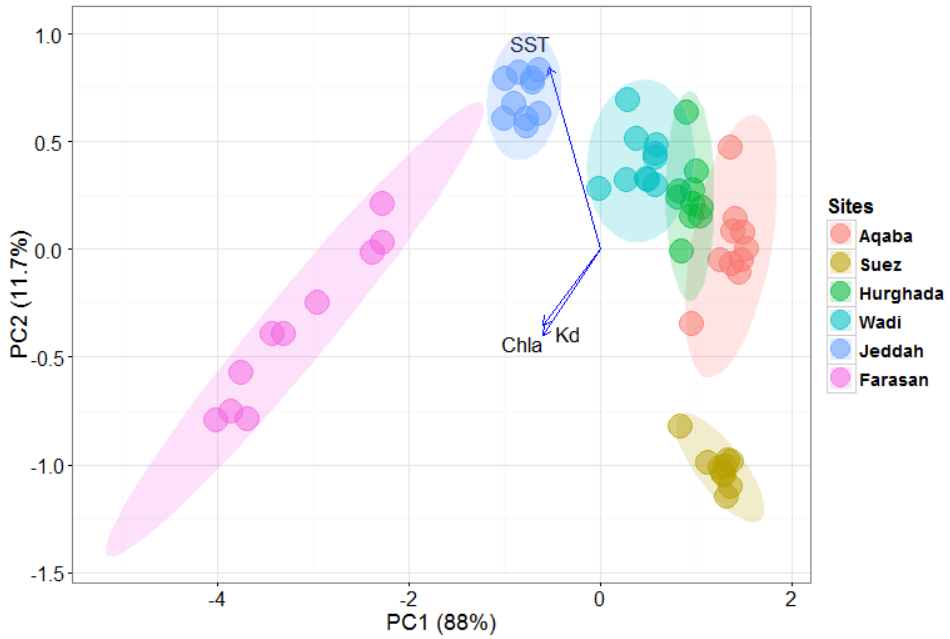

Figure **2-7**. Principle component analyses (PCA) plot of environmental variables (SST, Chlorophyll-*a* and K_d 490 nm). Data clustered and color coded by sites and each site represented by annual mean since 2003 to 2012. The major source of variation was PC1 (SST-88%) while PC2 (Chla) composed only 11.7% of the variation. The direction of loading for each parameter is indicated by the blue line, with the direction of the line pointed towards increasing values.

2.4.2. Bleaching pattern

Consistent with DHW across the Red Sea during 1982-2012, earliest reports of past bleaching records (i.e.1998 - Wilkinson, 2000) were restricted to the southern and central Red Sea (i.e. ca. 30% and 60% coral mortality was observed for southern Yemen/Sudan and Jeddah, respectively, see Table 2-3), while no bleaching was recorded in 1998 further north (e.g. Hurghada or Gulf of Aqaba, despite DHWs of >8°C-weeks for these sites) (Fig. 2-9). In contrast, the recent bleaching observations have been largely recorded from the central/northern Red Sea (Fig. 2-9), however, the northern Red Sea showed extreme thermal

tolerance toward high DHW. Specifically, Gulf of Aqaba and Egyptian Red Sea coast have not showed massive bleaching despite high DHW recorded (i.e. 15.1 Hurghada-2012, 10.9 & 13.4 Gulf of Aqaba and Wadi El Gemal respectively- 2010) except in Wadi El Gemal that showed 8-19% bleaching at 18.9 °C-week during 2012 (HEPCA, 2012). Overall, despite the apparent maximum DHW migration over the past two decades, northern sites appear to show high thermally induced bleaching resistance, with most recent incidents of bleaching restricted to the central Red Sea (Jeddah).

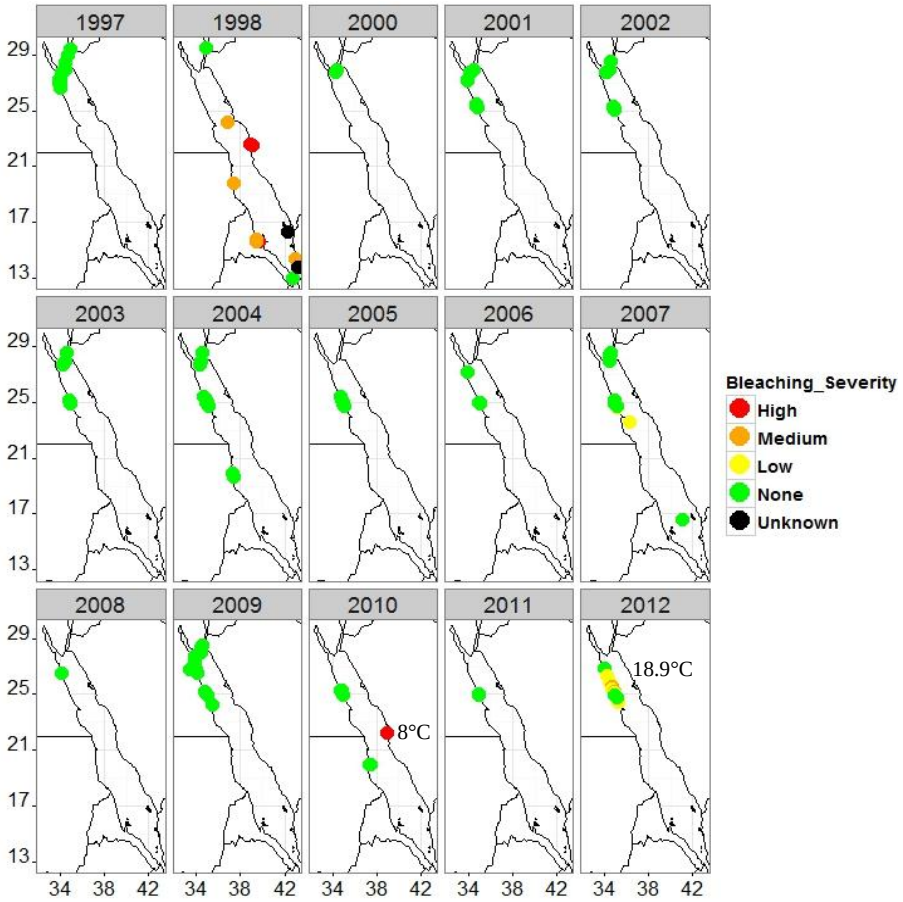

Figure **2-8**. Map shows geographical distribution and severity of coral bleaching events along the Red Sea during the period from 1997 to 2012. Bleaching severity data obtained from i) generous contribution from Blue Heaven Holiday diving center, Marsa Alam-Egypt, as reef check data conducted along Egyptian and Sudanese Red Sea coast. and ii) reefbase GIS database available for public use. It was clear that 1998 was the major event and affected mostly south -central of Red Sea; i.e. Saudi, Yemen, Djibouti and Eritrea with different intensity (bleaching with quantification data is symbolled as black dot), however no major effect on northern Red Sea. In contract, recent bleaching episodes were observed particularly in northern-central Red Sea (annotated by DHW), Egypt and Saudi during 2007, 2010 and 2012.

Together these various bleaching reports indicated that 42 genera (see Table S2 - Appendix) have been reported as bleached taxa; however, the most frequently reported were coral species within the genera of *Pocillopora*, *Acropora*, *Stylophora* and *Porites*, as well as the hydrozoan *Millepora* sp (see Table S1 - Appendix). Bleaching occurrences categorized by growth form surprisingly indicated that massive corals accounted for a greater proportion (45.4%) compared to branching genera (36.3%), and thus generally appear more thermally sensitive for this region. Both encrusting coral and soft coral genera showed relatively little susceptibility to bleaching (9 % each) (Fig. 2-9).

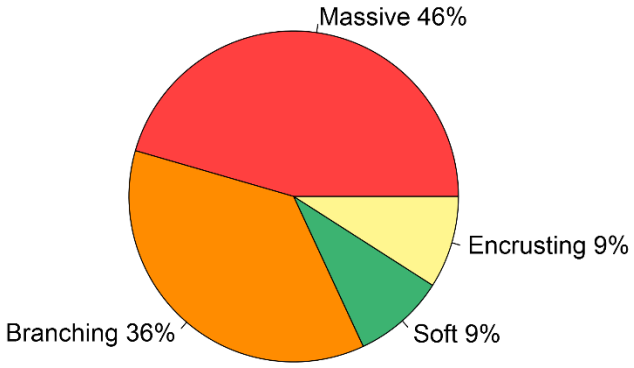

Figure **2-9**. Percentage of bleached coral species assigned to different growth form since 1998 to 2012. Data collected form literature, unpublished reports, reef check data and Reef-base database.

Table **2-3**. Summary of literature reports of coral bleaching events in the Red Sea since 1998 onwards

Country	Time	Status	Depth	SST/ºC	Reference
Egypt	1998	Egypt had insignificant mass bleaching however, some reports state that increasing water temperatures of shallow reefs (30-50 cm) has led to significant bleaching in several areas, but no quantitative data or reports have been collected or produced to support this. Surveys in 1999 reported a decline by 20-30% in coral cover but could not identify the reasons.	Worse affected corals (~50%) were at 2-5 m decreased to ~10% at 10 m depth	30 - 35 °C, particularly in shallow reef flat areas	(Pilcher & Abou Zaid, 2000; Wilkinson, 2000)
	2007	(1) Extreme low tides in March exposed the reef flats and resulted in coral bleaching and mortality. Wadi El Gemal was highly affected and recovered afterward (Mohamed Besar, Pers. Comm.) (2) Warm water bleaching event in October, with coral bleaching at 'Rocky Island' (~20m) where NOAA reported high DHW (12 °C-week according to Pathfinder 5.2) in the central Red Sea. Extent of coral mortality/recovery not quantified (Tamer Kamal & Mohamed Negm, Pers. Comm.)	20 m depth	----------	(Wilkinson, 2008)
	2012	Mass bleaching was recorded in southern Egyptian Red Sea when SST anomalies and DHW were higher than long-term averages. Bleaching ranged between 10 to 20% of total coral cover in seven of10 sites surveyed.	~50% were at 2-5m depth, decreased at 10m depth	32-33 ºC reached 34 ºC in some bays	(HEPCA, 2012)
Saudi Arabia	Aug 1998	Patchily distributed and highly variable bleaching in the central-northern Saudi Arabian extending from Jeddah to the Gulf of Aqaba. Most intense bleaching occurred near Rabigh (north Jeddah), where > 65 % of the total coral cover was bleached or recently dead (~20 - 40 % absolute cover). High coral mortality was observed along the southern Red Sea, where at some sites (i.e. Abalat Islands) live coral cover declined from 80 % in 1993 to about 10 % in 1999.	>50% occurred at < 6 m, but reached the base of the reef slopes (> 20 m depth)	> 32 °C three months prior to the first reports of coral mortality	(Devantier & Pilcher, 2000; Devantier et al., 2000)
	Aug-Sep 2010	Mass bleaching (~40%) occurred in central Saudi near Thuwal, where inshore reefs had a higher prevalence of bleaching (74 % of hard corals were bleached) than offshore reefs (14 % of hard corals), with highest bleaching prevalence at shallow reefs.	Dramatic bleaching was at 5 m, while decreased significantly at 10-15 m depth	10–11 DHW	(Furby et al., 2013)
Sudan	July 1998	Observations by scientists and divers reported corals were dead and covered with algal film at the western side of Sanganeb atoll when water level was exceptionally low, Bleached corals covered 14 % of the substrate (Nasr & Al-Sheikh 2000), however tourist operators indicated bleached corals in the southern Sudanese Red Sea could amount to 30 % of cover.	1-2 m depth	----------	(Pilcher & Nasr, 2000)
Yemen	1998	The Northern area of the Yemeni coast showed extensive coral bleaching particularly near Al Khawkhah, but there is no available quantifying data. Bleaching has caused extensive coral mortality on many Yemeni Red Sea	----------	>31ºC in May-June then cooling (< 24 ºC) in July	(Pilcher & Devantier, 2000;

		reefs, since 1990 to 1998 but major bleaching in 1998 was patchily distributed around the Socotra archipelago and NE Gulf of Aden.			Turak et al., 2007)
Eritrea	1998	Bleaching occurred on shallow and deep reefs, but most corals recovered after the temperatures dropped; there was some shallow water coral mortality. Anecdotal observations suggest significant coral bleaching around Massawa (Zekeria Abdul kerim, Pers. Comm.)	----------	40^{o}C	(Wilkinson, 1999)

2.5. Discussion

The assessment of environmental variability across reef ecosystems drives better understanding of reef structure, function, growth, diversity, morphology and health status (Gove *et al.*, 2013). The results of the current study demonstrate high degree of environmental variability/heterogeneity across the Red Sea, particularly within the southern Red Sea. Through the Red Sea, there is historical exposure to high SST and it has been suggested by Donner (2011) (see also; Brown *et al.*, 2002; Barshis *et al.*, 2013) that repeated exposure to anomalies results more heat-tolerant community. Fine *et al* (2013) proposed that high temperature at the southern entrance of the Red Sea acted as a selective barrier for heat-tolerant coral genotypes (>32 °C) recruited/colonized the Red Sea after the glacial age, and then dispersed larval/colonies over the Red Sea have a similar bleaching threshold (see Fine *et al*., (2013)). Accordingly, and due to thermal stress heterogeneity, this suggests that corals of the Red Sea should be able to tolerate anomalies to different extents. The observed localized thermal tolerance (this will be investigated in details in chapter 3&4) may result from small-scale differences in genetic composition of the holobiont through; i) acquiring/shuffling more thermally tolerant symbiotic algae (Berkelmans & van Oppen, 2006; Oliver & Palumbi, 2011a), ii) changing associated assemblage of microbes (Rosenberg *et al.*, 2007), or host physiological acclimation (Baird *et al.*, 2009).

The severity of annual anomalies of SST has appeared to increase northwards over past ~15 years and the values of the DHW observed are considered sufficient to cause bleaching-induced mortality [i.e. over the threshold of +1°C and accumulated over 8 °C week (e.g. Glynn 1991; Goreau & Hayes 1994; Liu *et al.*, 2006)]. In contrast, corals within the Red Sea seem remarkably tolerant of prolonged thermal stress (high DHW) perhaps reflecting an adaptation of the coral holobiont however, there has been some recent (e.g. 2012) mass

bleaching events within the northern Red Sea. The northern mass bleaching events may be a result of synergistic negative effect of high SST anomalies and high salinity (see; Edwards & Head 1986) which increase coral susceptibility to thermal stress (Nakano *et al.*, 1997; Kerswell & Jones, 2003). It is well reported that high or low salinity can disrupt the symbiotic relationship between zooxanthellae and coral host leading to bleaching (see; Goreau, 1964; Egana, 1982; Engebretson & Martin, 1994; Van Woesik *et al.*, 1995; Titlyanov *et al.*, 2000; Kerswell & Jones, 2003). Even so, the general trend of northward increase in both SST and thermal anomalies accompanied by bleaching is highlighting the importance of constructing regional thermal stress tolerance model.

Based on regional reports, Wilkinson (2000) suggested that Red Sea corals experience localized rather than mass regional bleaching. Research undertaken here suggests that both small and large scale environmental variability driven by the local and regional oceanography may underpin different levels of susceptibility across the Red Sea. Briefly - A pool of warm water develops in the central East Indian Ocean, gradually moved north reaching the southern Red Sea in late summer possibly inducing sporadic bleaching in southerly sites during 1998 (Turak & Brodie, 1999; Wilkinson, 2000). However, the northern Red Sea (Hurghada) showed high DHW (8.9, 11.3 and 15.1 °C-weeks at 1998, 2010 and 2012 respectively), no bleaching was observed.

This tolerance may be explained by two reasons; first, the influence of a South–North water current flowing along the eastern coast of Saudi, which sweeps around at the entrance of both Gulf of Aqaba and Suez to run North–South along Egypt (Eladawy *et al.*, 2015). This directional change creates clockwise eddies in the far northern Egyptian Red Sea coast associated with northerly wind at summer push water southward and hence, high current

speeds (cited in; (Fouda *et al.*, 1994) which could prevent bleaching by removing harmful stress-induced reactive oxygen species (ROS) (McClanahan *et al.*, 2005c; Nakamura, 2010). The effect of water current on coral tolerance to heat stress is supported by field observations from the Seychelles and Indonesia (see West & Salm 2003).

The second possible hypothesis based on the data is that high frequency and intensity of DHW incidents (see Figure 2-5) may build up thermal tolerance in this region and enhanced holobiont resistance toward thermal stress. It has been reported that coral reefs with naturally higher temperature variability and frequently exposed to short-term stress-inducing temperatures may lead to greater tolerance to prolonged thermal stress (Castillo & Helmuth, 2005; Oliver & Palumbi, 2011a; Howells *et al.*, 2016). The tolerance may suggest thermal acclimation over time (Maynard *et al.*, 2008), corals shuffled *Symbiodinium* to heat tolerant clade (Baker *et al.*, 2004; Berkelmans & van Oppen, 2006), energy reserve and heterotrophic plasticity to compensate insufficient photosynthesis outcome (Fitt *et al.*, 2000; Grottoli *et al.*, 2004) and mortality of vulnerable species that could not re-populate after heat stress events (Thompson & van Woesik, 2009). Advanced genomic techniques explained the capability of corals inhabiting extreme environment to express heat related genes in the regulation process to mitigate the effect of thermal stress (DeSalvo *et al*., 2010; Barshis *et al*., 2013). The tolerance of corals at the north Red Sea (Hurghada) highlights the possible global value of this area as it concerns a genetic stock of tolerance corals. It also highlights the need for more expansive and detailed research into the environmental and genetics drivers of coral tolerance (see chapter 3&4), the need for further study to examine its capability to work as a refuge habitat such as Gulf of Aqaba as reported by Fine *et al* (2013).

Observed bleaching in 2007, at Wadi El Gemal, was associated with SST anomalies (DHW 12.3°C-week) combined with low water levels, but no mass regionally-wide bleaching occurred and impacts were localized (e.g. particularly at Rocky Island and National Park camp, Mohamed Besar & Mohamed Negm, per. comm.). Photo-investigation of bleaching during 2012 at Wadi El Gemal (see; HEPCA, 2012) revealed that the prolonged thermal stress (DHW 18.9°C week) induced widespread bleaching associated with white plague disease. The correlation between thermal stress and high prevalence of coral disease causing mortality is well documented (Gil-Agudelo & Garzón-Ferreira, 2001; Kuta & Richardson, 2002; Boyett *et al.*, 2007; Bruno *et al.*, 2007) by increase pathogen virulence or decrease host disease resistance (Harvell *et al.*, 2002). Accordingly, it is not surprising therefore that mass bleaching events and disease outbreaks have been linked temporally during thermal anomalies (Miller *et al.*, 2011). This is highlighting the importance of monitoring the prevalence of coral disease during summertime/bleaching events due to the link between anomalous SST and disease outbreak (Borger & Steiner, 2005) .

There are clearly strong relationships between thermal anomalies and bleaching episodes, but this relationship may mask other factors that influence bleaching sensitivity. Chlorophyll-*a* has been used as an indicator of stress inducing environmental conditions for coral reefs (Maina *et al.*, 2011), serving as a proxy for eutrophic conditions. Nutrification can increase the thermal susceptibility of corals (see; Fabricius *et al.*, 2013) through; i) altering thylakoid membrane structures (Wiedenmann *et al.*, 2012), ii) producing more harmful oxygen radicals due to high zooxanthellae density (Cunning & Baker, 2012) iii), reducing the photosynthetic efficiency and light-harvesting capacity (Dubinsky *et al.*, 1990), and iv) absorbing more light energy as darker colony due to high photo-pigments and hence more

stress (Fabricius, 2006). In contrast, nutrification may increase tolerance of corals by; i) reduce the light dependency of corals (switching to heterotrophy, see Grottoli et al. 2006), ii) acquiring clade D symbiont that usually found in turbid-low light water at Indo-pacific coral reefs (Van Oppen *et al.*, 2009), and iii) increase the recovery rate by using/burning stored energy reserve (Fitt *et al.*, 2000; Grottoli *et al.*, 2004). In addition, high Chl-*a* often correlates with high turbidity, which can reduce light penetration creating a shading effect, and then reduce the combined stress impact of high temperatures and light (Mumby *et al.*, 2001; Suggett & Smith, 2011). Overall high chlorophyll content is generally considered an indicator of poor water quality and pollution, which reduces coral resistance to other stressors (e.g. SST) (Goreau *et al.*, 2000; Maina *et al.*, 2008).

Spatial variation in Chl-*a* and K_d in the southern Red Sea is partly driven by the exchange of less saline, colder, nutrient enriched and turbid water with Gulf of Adan through the Bab El-Mandab straits, in addition to upwelling of bottom current that collide by 200 m depth stair at Bab El-Mandab (Morcos, 1970; Maillard & Soliman, 1986). This water exchange occurs during summer months and then reverses to flow into Gulf of Adan, and explains the significant fluctuation (hence ±SD of annual mean) of Chl-*a* and K_d within the southern Red Sea and the gradual reduction northwards (see, Figure 2-6). Thus is appears that the obvious southern Red Sea SST decline is a result of the combination of water exchange and upwelling (see Figure 2-2).

Identification of the taxonomic variation in bleaching susceptibility is an important outcome of this work. Our analyses of previous reports along the Red Sea have demonstrated that massive coral species are more susceptible to thermal stress than branching corals and this hypothesis will be tested specifically in chapter (4). Field observations of the Western

Indian Ocean did not support this finding and showed branching taxa (such as *Acropora* and *Pocillopora*) to be more susceptible to thermal stress than massive taxa such as *Porites* and some Faviids (e.g. West & Salm 2003; Mcclanahan et al. 2007). Such contrasting patterns in the Red Sea may be related to the high relative abundance of massive corals and may also reflect size-based difference (Loya *et al.*, 2001; Bena & Van Woesik, 2004) as was evident among *Pocillopora* corals (Guest *et al.*, 2012). The general pattern observed here may be explained by differences in mass-transfer capacity, whereby flatter and smaller corals have a greater capacity to remove potentially deleterious superoxides and other oxygen radicals, compared to more erect and branching forms (Nakamura & Van Woesik, 2001). However, such generalities are not currently supported by empirical data (Baird & Marshall, 2002), thus emphasizing the need for more research focused on the susceptibilities of individual coral colonies as well as species and most importantly the "holobiont" which will be the focus in chapter 4.

In conclusion, the findings highlight the heterogeneity of environmental conditions across the Red Sea with obvious magnitude trend of SST anomalies and DHW at the northern Red, leading to considerable coral bleaching, particularly at southern Egyptian coast and central Red Sea. Regardless the thermal stress at far north Egyptian Red Sea coast (Hurghada, 15.1 °C-week in 2012), it remained bleaching free, which highlight that this area may be considered "pockets of resistance". Understanding the acclimation mechanism of Red Sea corals to thermal anomalies would be a major step forward to understand those mechanisms that may increase tolerance in the future and will help address the key question of whether or not systems that have experienced thermal anomalies in the past are more or less at risk when compared to other sites. The next chapter considers how changes in genetic

composition at the level of the coral holobiont (i.e. microbiome diversity) potentially contributes to such processes of acclimatization throughout the northern Red Sea.

Chapter 3

3. *The composition of coral microbiome at different locations across the northern Red Sea*

3.1. Abstract

The Red Sea is inhabited by one of the best growing and healthy coral reefs in the west Indian ocean however, it has extreme conditions such as high sea surface temperature, salinity and light intensity. Adaptation to such 'present day' extremes make the Red Sea a perfect model to understand coral tolerance to future stressors, notably global warming (i.e. thermal stress) that is increasingly impacting coral reefs worldwide. The current study investigates the microbiome (i.e. *Symbiodinium* spp. and microbial communities) associated with corals along different locations of the Egyptian Red Sea to determine for the first time the natural plasticity/variance of the microbiome in response to different thermal histories (>3°C in SST summer mean). Samples collected from six coral species at five sites and two depths (total n=163) revealed that the composition of microbiome did not vary among two depths. *Symbiodinium* assemblage was dominated by clade C ITS2-types (13 subclades), and was conserved between sites and depths however, it exhibited a high level of host-symbiont specificity at subclade level. Conversely, the microbial community (as determined through 16S metagenomics) differed significantly between sites and coral species however, only 11 OTUs composed 52% of total abundance of the microbial community. To test microbial specificity, analysis of indicator species revealed that ca. 30% and 15% of total OTUs were specific to sites and coral species respectively and represented a broad range of total relative abundance (10% to 62% for sites and 6%-64% for species). Noticeably, the specific microbial community associated with coral species was mainly dominated by Order Oceanospirillales that always associated with healthy corals, whereas those specific to sites were belonging to

different taxa. Interestingly, corals from the southernmost site were dominated (6 fold higher than northernmost site) by rarely reported *Erythrobacter* sp. This group contains bacterial chlorophyll-*a* and carotenoids that may provide the host with a supplementary photosynthetic source. This work shows for the first time the composition of microbiome across different thermal regimes suggesting that the microbial consortium other than *Symbiodinium* drives the variability of the coral holobiont (i.e. non-zooxanthellae microbiome), whilst *Symbiodinium* spp. have a broad thermal niche allowing the dominance of a single subclade across this region. This suggest that the adaptability of the coral microbial community may be a key mechanism to enable corals to populate different thermal histories.

3.2. Introduction

Coral reefs experience rapid decline in biodiversity due to increasing the frequency and intensity of thermal-induced mass bleaching (Hoegh-Guldberg *et al.*, 2007; De'ath *et al.*, 2012). Thermal anomalies reduce the potential resistance of corals and magnitude of the breakdown of host-algal symbiotic relationship (Wooldridge, 2013) which reduces the host's ability to meet its metabolic needs, and hence mortality (Wooldridge, 2010). Therefore, the major challenge facing corals worldwide currently is rapid increase of water temperature, and hence thermal anomalies, that are predicted to continue rising by 2-3°C by end 21th century (IPCC, 2014). Fortunately, corals can adapt/acclimate to thermal anomalies to different extents however, the mechanism remains yet not well understood (Middlebrook *et al.*, 2008; Weis, 2010; D'Angelo *et al.*, 2015).

Thermal tolerance of reef-building corals is mainly governed by the genetic make-up of coral holobiont (Rohwer *et al.*, 2002) comprised from coral host and its associated *Symbiodinium*, archaea, fungi, endolithic algae and bacterial community, where some of those microorganisms are host-specific (Ritchie & Smith, 1997a; Koren & Rosenberg, 2006;

Carlos *et al.*, 2013). The sum of all associated microorganisms, which defined as the "coral microbiome", is playing vital roles in corals health and its resistance towards environmental stressors (Rosenberg *et al.*, 2007; Kelly *et al.*, 2014; Ainsworth *et al.*, 2015). The microbiome is an important partner for the coral host where it performs photosynthesis, nitrogen and carbon fixation, mineral recycling that all lead to provide nutrients to the host (see; Bourne *et al.*, 2016). Therefore, the coral holobiont can locally adapt to specific thermal regimes via changing the microbiome community through; i) selection of more heat stress tolerant *Symbiodinium* spp. (e.g. Lajeunesse *et al.*, 2010; Howells *et al.*, 2011; Oliver & Palumbi, 2011a; Silverstein *et al.*, 2015) or by ii) changes to the microbial community (Reshef *et al.*, 2006; Kelly *et al.*, 2014).

Several studies have confirmed that the genus *Symbiodinium* is a highly diverse group (Baker, 2003; Lajeunesse *et al.*, 2012; Wham & LaJeunesse, 2016) that are likely to be distinct physiologies, some of which may mitigate the effects of coral bleaching conditions (Van Oppen *et al.*, 2009; Stat *et al.*, 2013). For example, it has been reported that corals harbor clade A have limited photo-acclimation capability (Iglesias-Prieto & Trench, 1997), while those have clade B are more tolerant to high light intensity (Secord & Muller-Parker, 2005). Also, *Acropora* and *Pocillopora* spp. hosting clade D exhibited higher thermal tolerance than corals harbor clade C type (Rowan, 2004; Jones *et al.*, 2008; Stat & Gates, 2011). Recently, Hume *et al* (2015) identified a novel *Symbiodinium thermophilum* that survive in extreme warm water (>35 ^{o}C) within the Arabian Gulf. This highlights that different symbionts clade (and subclade) types provide different physiological and ecological benefits for coral hosts, which emphasize on the necessity to identify symbiont genetic

variants, particularly in extreme environments (see; D'Angelo *et al.*, 2015; Howells *et al.*, 2016).

Moreover, recent studies revealed also that thousands of distinct bacterial phylotypes are associated with individual coral colonies (Lee *et al.*, 2012; Bayer *et al.*, 2013a), which are increasingly recognized as contributors to mediate the effects of environmental stressors (Kelly *et al.*, 2014; Ainsworth *et al.*, 2015; Röthig *et al.*, 2016). Therefore, the taxonomic profile of the microbial community should be considered because it refers to different functional role of corals holobiont (Gates & Ainsworth, 2011; van de Water *et al.*, 2016). For example, Ainsworth *et al* (2015) identified ubiquitous endosymbiotic bacterial phylotypes which facilitate dinoflagellate endosymbiosis (see also; Neave *et al.*, 2016). It has been documented that the microbial community differs within corals holobiont compartments (i.e. surface mucus, tissue, skeleton microbes) (Sweet *et al.*, 2011a), but coral surface mucus has the key role in mediating coral health (Glasl *et al.*, 2016). Its importance not only stems from its nutritional, protective and cleaning role (Brown & Bythell, 2005), but also its ecological function through its rapidly dynamic (adaptive) nature to environmental stressors and defensive role by production of antimicrobial substances (Rosenberg *et al.*, 2007; Shnit-Orland & Kushmaro, 2009).

The relationship between coral host and its symbiotic algae (i.e. *Symbiodinium* spp.) is fairly well studied worldwide (Rowan, 2004, see also LaJeunesse & Thornhill, 2011; Thornhill *et al.*, 2014). Also, growing research is carried out on microbial communities associated with corals (Ritchie & Smith, 1997b; Mills *et al.*, 2013; Ng *et al.*, 2015; van de Water *et al.*, 2016), but the Red Sea remains yet not well explored. Few studies have characterized *Symbiodinium* in the Red Sea, particularly at Saudi Arabia and far north Gulf

of Aqaba, Eilat (see; Barneah *et al.*, 2004; Baker *et al.*, 2005; Sawall *et al.*, 2014; Ziegler *et al.*, 2015b, see also chapter 1, pp 35). However, these attempts highlighting some spots along the Red Sea, the high degree of environmental variables across the Red Sea that really affect *Symbiodinium* distribution yet to be examined.

Similar to *Symbiodinium*, microbial community associated with corals is poorly studied across the Red Sea however, there are a few localized studies have been carried out to investigate the bacterial community associated with corals and their response to environmental stressors. Lampert *et al* (2006) showed high heterogeneity of the bacterial community within mucus associated with *Fungia scutaria* (Gulf of Aqaba), while Lee *et al.*, (2012) showed that bacterial community, collected from Thuwal- Saudi Arabia, was specific to coral species that was also influenced by environmental variables (i.e. depth & salinity) among sites. Jessen *et al* (2013) reported that two *Endozoicomonas* spp. dominated two thirds of the bacterial community associated with *Acropora hemprichii* during eutrophication and overfishing stress experiment. Likewise, Bayer *et al* (2013) found that *Endozoicomonas* was highly associated with *Stylophora pistillata* collected from Saudi Arabia and proposed that it has an intimate relationship with corals. Recently, Ziegler *et al* (2015) reported significant microbial shifts among corals inhabiting impacted sites (local sewage and discharge of waste water) in Jeddah, Saudi Arabia. Also, Röthig *et al* (2016) showed significant shifts in microbial communities associated with *Fungia granulosa*, to be dominated by *Pseudomonas veronii* after exposure to long term brine water (55‰) at Thuwal, Saudi Arabia.

Whilst these studies are important to show emerging patterns from the Red Sea, no research has yet conducted on Egypt's Red Sea coast, the location that experiences extreme thermal stress (i.e. DHW >15 °C-weeks), but remains bleaching free (see chapter 1). This

research is highly required to determine how the composition of the microbiome varies across different coral species and the same coral species but from different sites experience different thermal conditions. Therefore, the aim of this chapter was to investigate the composition of the microbiome community (i.e. *Symbiodinium* and microbial community) of corals' inhabiting different latitudes of the Egyptian Red Sea coast. It is important to understand the composition of microbiome associated with corals exhibiting high thermal tolerance, and its reorganization over different sites experience different thermal regimes.

The hypothesis of this chapter is that thermal tolerance may be linked to prevalence of *Symbiodinium* variant or specific microbial phylotypes that improve thermal tolerance of corals. Also, it proposed that the microbiome will change in the composition across sites as a response to thermal stress that increases southwards. Hence, the objective is to investigate *Symbiodinium* clade types and microbial communities associated with coral species inhabiting different thermal regimes. To achieve the objectives chapter of this chapter, microbiome communities associated with six coral species across five sites experience different thermal histories were identified across the Egyptian Red Sea coast. This study fills a gap of knowledge and conduct, for the first time, genetic analysis on the microbiome associated with corals to improve our understanding of the spatial structure of the microbiome across corals species and thermal regimes in the Red Sea.

3.3. Materials and Methods

3.3.1. Survey sites and samples collection

*Survey sites*__ The Egyptian Red Sea coast is approximately 1000 km long and stretches from the far north of Gulf of Aqaba to the Sudanese boarder in the south. The survey was conducted in February-2013 along Egypt's Red Sea coast at five sites to represent covering north Gulf of Aqaba to south Egyptian Red Sea coast. Sites specifically were Abo Galloum (28.6147°N, 34.5604°E) at Gulf of Aqaba, Ras Mohamed (27.7305°N, 34.2691°E) at Saini peninsula, (3) Abo Galawa (27.3157°N, 33.8097°E), Meritte (27.2485°N, 33.849°E) at Hurghada, and Wadi El Gemal (24.6988°N, 35.1327°E) (Fig. 3-1). All sampling sites were fringing reefs adjacent to the shoreline, expect Abu Galawa which was patchy coral and ~1 mile away from the coast. Also, all sites were not heavily populated and have clear water, except Meritte which was a turbid site as a result of land-fill, as well as it was impacted by heavy touristic activities (snorkeling, diving, artisanal fishing and fish feeding).

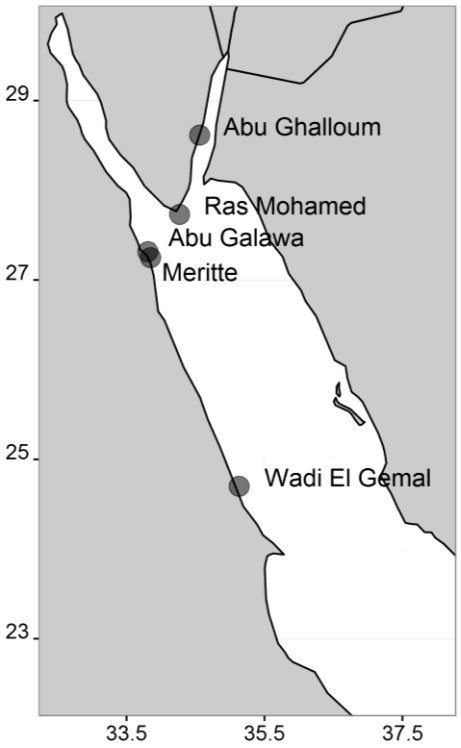

Figure **3-1**. Map of the study sites along the Egyptian Red Sea located across latitudinal gradient ranged from Gulf of Aqaba to Wadi El Gemal at the south of Egyptian Red Sea.

*Sample collection*__ At each site, six coral species were chosen to represent different coral growth forms commonly recorded across the Egyptian Red Sea coast, specifically; massive *(Porites nodifera* and *Favia favus*), branching (*Pocillopora damicornis* and *Seriatopora hystrix*); the soft corals *Xenia umbellate* and *Sarcophyton trocheliophorum*, were

also sampled. All samples were collected from two depths (shallow from 2-5m and deep from 15-18m). At each site, three types of samples were collected as follows;

1-Coral fragments (<1 cm, n=3) were collected from healthy colonies (>5 m apart) for each coral species at two depth levels using SCUBA diving. All coral fragments were collected using a hammer and chisel, cutter and knife/scissors as needed for each coral species. All samples were placed in separate pre-labeled zipped plastic bags filled with in situ seawater. Upon return to the laboratory (within 2 hours), all coral samples were preserved in preloaded 2 ml cryovail containing DMSO-20% buffer (0.25M disodium EDTA pH8, NaCl saturated, 20% Dimethyl Sulfoxide, see Seutin et al., 1991) for DNA preservation and subsequent *Symbiodinium* identification (using ITS2).

2- At each sampled coral colony, associated surface mucus was sampled using sterilized 50 ml syringes which were then kept shaded in a cold box till return to the lab.

3- Seawater samples (500 ml) were collected at each site (n=3) at each depth level in sterilized polyethylene bottles at >5 m distance from the sampled reef/colonies as a reference microbial samples for each site.

Upon return to the laboratory, each mucus and water sample was filtered through sterilized 0.22 µm Cyclopore filter column (Whatman, UK), and then preserved in 2 ml vials preloaded with DMSO-20% buffer solution for 16S microbial analysis. All preserved coral fragments and bacterial samples were kept in DMSO-20% vial at room temperature, and then in the fridge at 4 ^{o}C (see Gaither *et al.*, 2011) till shipping into UK, University of Essex for genomic analysis.

3.3.2. *Symbiodinium* ITS2 clade identification

DNA extraction— Nucleic acids were extracted using the Wizard DNA prep protocol by Promega (Madison, WI, USA). Briefly, small fragments of coral tissue (ca. 30–40 mm^2

surface area) were placed into 1.5 ml micro-centrifuge tubes with 250–350 mg of 0.5 mm glass beads and 600 µl of Nuclei lysis buffer (0.2 M Tris, 2 mM EDTA, 0.7% SDS, pH 7.6) and bead-beaten for 1 min at 1200 g in a Bead beater. The lysate was then incubated with 3 µl proteinase K (20mg/ml) for 1 h at 65°C in heat block, and then 250 µl protein precipitation buffer (9M Ammonium Acetate) was added and the extract was chilled on ice for 15–20 min. After centrifugation for 5 min at 13000 rpm, 600-650 µl of supernatant was transferred to new 1.5 ml tube containing 700 µl of 100% isopropanol and 25 µl of sodium acetate (3M). Tubes were chilled at -20 °C for >20 min, and then DNA was precipitated after being centrifuged (13000 rpm) for 5 min. Isopropanol supernatant was removed and the pellet washed twice with 500 µl of 70% ethanol (EtOH) then centrifuged again for 5 min (13000 rpm), dried, and re-suspended in 80 µl of DNase free H_2O.

PCR-DGGE— Due to the variability of the internal transcribed spacer 2 region (ITS2), it is presently the prominent useful genetic marker for distinguishing *Symbiodinium* clades and subclade. Using genomic DNA, *Symbiodinium* ITS2 region was amplified to produce 330–360 bp amplicon product using two stages;

1-Nested PCR (10 µl total reaction) was carried out for 18S and 28S-rDNA genes to maximize *Symbiodinium* specificity, using primers designed by Santos *et al* (2001) mixed with 1 µl gDNA and Dream-Taq polymerase (ThermoFisher). Nested PCR thermocycler protocol was as the following: 95°C for 5 min initial denaturation temperature, 45 cycles as 95°C for 30 sec for DNA denaturation, 52°C annealing temperature for 30 sec, 45 sec extension time at 72°C, and final extension time at 72°C for 10 min.

2- The ribosomal ITS2 (25 µl reaction) was then amplified using 1 µl of nested PCR amplicon and 1:3 ZITS2 forward to reverse (DGGE clamp underlined) primers ratio.

Forward primer sequence was 'ZITS2for' (5′GAATTGCAGA ACTCCGTG 3′) and reverse primer with GC clamp was 'ZITS2 clamp' (5′ CGCC CGCC GCGC CCCG CGCC CGTC CCGC CGCC CCCG CCCG GGAT CCAT ATGC TTAA GTTC AGCG GGT 3′) according to LaJeunesse & Trench (2000). A ''touchdown'' amplification protocol with annealing conditions 10°C above the final annealing temperature of 52°C was used to ensure PCR specificity. The annealing temperature was decreased by 0.5°C after each of 20 cycles. Once the annealing temperature reached 52°C, it was maintained at that setting for another 20 cycles.

ITS2 amplicon was tested in 1% agarose gel electrophoresis at ~90 mV for 30 min. Gel was stained by Ethidium bromide solution (5 µl) and investigated under UV light. Successful ITS2 amplicon were loaded on to 8% polyacrylamide gradient gel electrophoresis (gradient of 45–80% urea and formamid polyacrylamide gel) at constant 60°C for ~15h (LaJeunesse, 2002) using a CBS Scientific System (Del Mar, CA, USA). Samples loaded aligned to mixture of pre-identified *Symbiodinium* clade (B1, C1, D1) as a reference ladder. DGGE gels were stained by 2 µl SYBR green (Molecular Probes, Eugene, OR, USA) mixed with 10ml 1X TAE buffer for ~30 min, and then photographed using a Fotodyne (Hartland, WI, USA) imaging system under UV light.

Sequence preparation— Different fingerprinting bands for each coral species/site were excised and placed separately in 1.5ml Eppendorf tube containing 0.5ml of RNase free water and stored at 4°C overnight. Later, re-amplification of excited bands was performed using 1:1 ZITS2 forward and reverse primer without GC clamp in 15 µl PCR reaction using the following thermocycler protocol; initial denaturation: 92 °C/3min, denaturation and 40 cycle at 90 °C /30 sec, annealing: 52°C/40 sec, extension: 72°C/30 sec and final extension at

72°C/10 min. After testing the ITS2 amplicon in 1% agarose gel, 4 µl were mixed with to 1.5 µl of USB-EXO SAP-IT PCR cleanup kit (affymetrix, USA) and placed in thermocycler for 37°C for 15 min and 80°C for 15 min to clean/remove excess of primers and nucleotides of ITS2 amplicon. The product was mixed with 16 µl of RNase free water, and then 10 µl were taken and mixed with 5 µl of ITS2 forward and reverse primer (5M) separately. Reaction products were analyzed using Sanger sequencing on an Applied Biosystems 310 genetic analyzer, USA.

3.3.3. Bacterial 16S metagenomics

DNA extraction— Seawater and mucus filters were used to extract bacterial genomic DNA using the CTAB (Cetyl-trimethyl-ammonium-bromide) method (Griffiths *et al.*, 2000) for 16S metagenomics library preparation. Briefly; filters were placed in sterilized 2 ml screwed cap vials preloaded with 0.6 ml CTAB buffer (0.7M NaCl, CTAB), 0.5 ml Phenol:Cholroform:Isoamyl alcohol (25:24:1, pH 8) and 0.5 g sterilized silica beads. After samples were bead-beated for 30 sec at 2000 rpm and cooled in ice, samples were centrifuged (5 min at 13000 rpm) and the supernatant were taken and placed in new sterilized 1.5 ml Eppendorf tubes preloaded with 1 ml of PolyEthelen Glycol/PEG (6000/1.6M NaCl) that had been inverted several times and left at room temperature overnight. Samples were then centrifuged (5 min at 13000 rpm), then DNA pellets were washed twice with 70% ethanol, then re-centrifuged and re-suspended in 60 µl of sterilized milli-q water. DNA quantity and quality were assessed on a NanoDrop ND 1000 spectrophotometer (Thermo Scientific, DE, USA).

Bacterial 16S library preparation— To amplify bacterial 16S rRNA gene of mucus and water samples, hypervariable V3 and V4 regions of ribosomal DNA were targeted (~550pb) using 805 reverse primer 5' GTCT CGTG GGCT CGGA GATG TGTA TAAG

AGACAG GACT ACHV GGGT ATCT AATCC 3' and 341F forward primer 5' TCGT CGGC AGCG TCAG ATGT GTAT AAGA GACAG CCT ACGG GNGG CWGC AG 3' attached with Illumina overhang adaptor (underlined, Illumina, San Diego, CA, USA). For each sample, 25 µl PCR reaction was performed using 1 µl of bacterial gDNA and 5 µl of each primer (1 µM) mixed with REDTaq ReadyMix (Sigma-Aldrich, UK) and RNase free water. PCR thermocycler protocol was at 95°C/3 min for initial desaturation, then amplified for 32 cycle at 95°C/30sec, 56-60°C/30sec (adjusted to minimize primer dimer) annealing temperature, 72°C/30sec, and final extension at 72°C for 5 min. PCR 16S amplicon (5µl) were visually checked on 1% agarose gel electrophoreses (~110 V for 30 min) after staining in Ethidium bromide solution (200 µM EtBr in 500 ml TAE buffer) for 30 min.

Successful PCR amplicon (remaining 20 µl) was transferred to a 96 well plate, and then cleaned by AMPure XP magnetic bead system (Beckman Coulter, Brea, CA, USA). Afterwards, 5 µl of cleaned PCR amplicon were used for indexing PCR for 28 cycle using Nextera XT V2 kit (A&B index kit) (Illumina) according to the manufacture manual. Indexed PCR amplicon was cleaned again by AMPure XP magnetic beads and then quantified using FLUOstar Omega microplate reader (BMG Labtech, Germany) using Quant-iT PicoGreen dsDNA assay kit (Invitrogen, USA), and then all samples pooled in equimolar ratios. The quality of the final pooled library was checked on Bioanalyzer (Agilent 2100, Santa Clara, CA, USA), and the library then sequenced on the Illumina HiSeq platform, 2x 300pb paired end by Version 3 chemistry kit at TGAC genomic analysis center (Norwich, UK).

Bacterial bioinformatics Analysis — Quality control of sequence data was performed as described in Schirmer *et al* (2015). Raw sequences were first quality trimmed using Sickle version 1.33 (Joshi & Fass, 2011). Sequences were trimmed at the default quality threshold

(Q20) using paired-end mode, and all sequence with ambiguous bases (Ns) were discarded. The sequence trimming carried out at 3' end of the sequence, and then length threshold of 250pb was set up to discard shorter reads. Pairs of sequences that passed previous steps were then subjected to error correction using BayesHammer implemented SPAdes v3.7.1, with default settings (Nikolenko *et al.*, 2013; Nurk *et al.*, 2013). Forward and reverse reads were then paired-end aligned and primers removed using the PEAR algorithm implemented in PANDAseq version 1.33 (Masella *et al.*, 2012; Zhang *et al.*, 2014). Paired reads were then de-replicated, sorted by abundance and clustered into operational taxonomic units (OTUs) at 97% similarity threshold using Vsearch v1.11.1 (Rognes, https://github.com/torognes/vsearch). Low abundance sequences (<5 occurrence) that more likely representing erroneous sequences were removed. Taxonomy was assigned to OTU centroids using the RDP classifier (Wang *et al.*, 2007) as implemented in Qiime (Caporaso *et al.*, 2010), with a minimum 0.7 confidence level and relative abundances of taxa were computed using Qiime's "summarize_taxa.py" script. All bioinformatics analyses were conducted using the Bio-Linux 8 operating system.

3.3.4. Data Analysis

All DGGE gels of *Symbiodinium* were assessed visually first to identify the fingerprint for each sample as per LaJeunesse *et al* (2003). Afterwards, all sequences of *Symbiodinium* bands were first trimmed (Geneious V8) to remove sequence noise and primers, and then aligned/blasted against Dr. Eugenia Sampayo (University of Queensland, Australia) and Prof. Todd LaJeunesse (Pennsylvania state University, US) *Symbiodinium* database for clade identification. Thereafter, data was tabulated and transformed into presence/absence data matrix for statistical analysis. To test the significance of similarity of

Symbiodinium community between sites, coral species and depth, Analysis of Similarity test (ANOSIM) (Clarke, 1993) was performed using present/absent data matrix.

For microbial community analysis, OTUs abundance matrix was used for all statistical analysis. Microbial diversity indices (i.e. Chao1 richness estimator, Inverse Simpson and Shannon diversity indices) were calculated for each sample separately (total n=168). Normality of diversity indices were checked using Shapiro test (Shapiro & Francia, 1972), and then data normalized by log or square root transformation if needed. Thereafter, the influence of sites, corals and depth on microbial diversity were assessed using multifactorial ANOVA. Principle Coordinate Analysis (PCoA) ordination plot, using Bray-Curtis dissimilarity, was used to visualize the dispersion of microbial community among sites, coral species and depth.

Multivariate analysis was further used to test the statistical difference of microbial community structure. Permutation multifactorial Analysis of Variance (PERMANOVA) (Anderson, 2001) with 999 permutations using Bray-Curtis dissimilarity using *'adonis'* function in "R" PERMANOVA was performed first on i) all coral samples to assess the influence of sites, corals and depth and their interactions on microbial community structure, ii) each coral species across sites (e.g. *F. favus* across five sites) to investigate the effect of site and on each coral species separately, iii) each site include all coral species (i.e. all corals within each site) to assess the influence of coral species on microbial composition at each site separately. To assess the statistical difference of similarity among factors, Analysis of Similarity (ANOSIM) (Clarke, 1993) was performed on OTUs abundance matrix using Bray-Curtis dissimilarity matrix. To identify the OTUs that significantly associated ($p<0.05$) with each site and coral species, Indi-species package in "R" (Cáceres & Legendre, 2009) was

used, that returned a list of OTUs associated with each studied factor. All plots and statistical analysis were performed in "R" version 3.2.3 (R Developement Core Team, 2015)

3.4. Results

3.4.1. *Symbiodinium* assemblage structure

Genetic characterizations of six coral species (n=163) along the surveyed sites in both shallow and deep water identified a total of 17 distinguishable *Symbiodinium* ITS2 types belonging to clade A, C and D (see DGGE fingerprint in appendix; Figure S4-S9). Analysis of PCR-DGGE of ITS2 revealed that clade C was the most prevalent symbiont type (ca.85%, n=140) and composed thirteen subclade (C1, C1 variant, C15, C15n, C15p,o, C170, C170a, C171, C1h, C3z, C41, C65 varian1, C56 variant2,) out of the 17 recorded types (Fig. 3-2). Thirteen percent of host species harbored clade A type (13%) comprised of three subclade types (A1, A1c, A1 variant), whilst only 1% of hosts (i.e. *X. umbellate*) harbored D3 variant (Fig. 3-2 and 3-3).

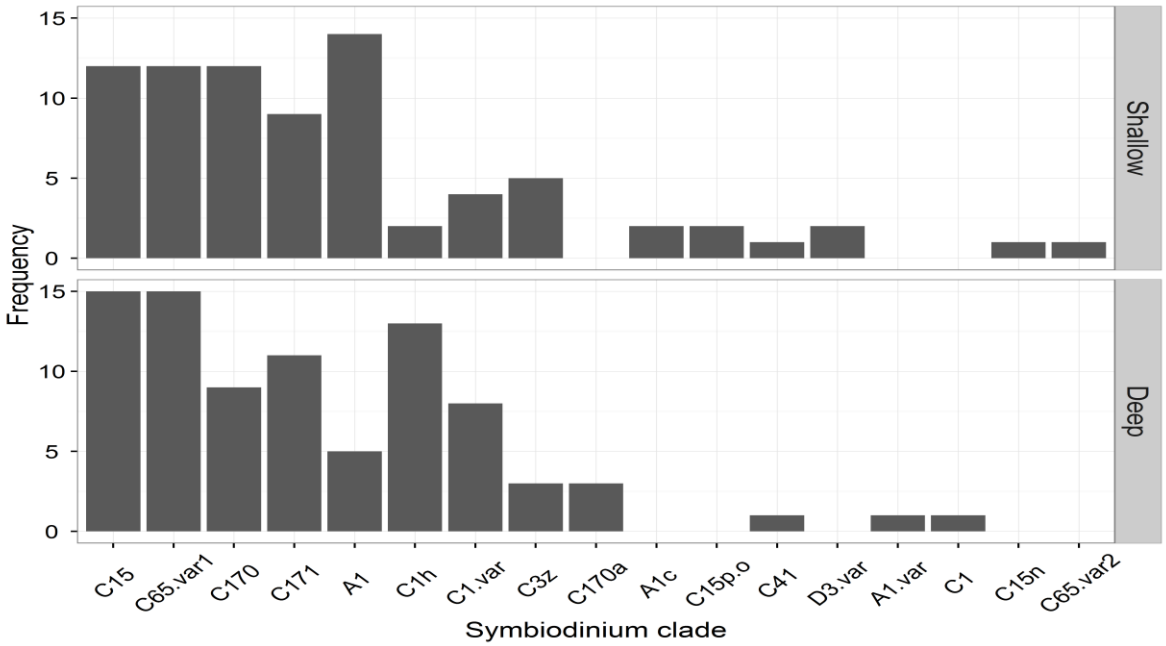

Figure **3-2**. *Symbiodinium* clade ITS2-types frequency (n=163) of six coral sampled species along different five sites at two depth levels. The plot demonstrates the occurrence of 17 clade types belonging to three clade types, with high frequency of clade C and occasional presence of clade A and D. It is clear that no difference *Symbiodinium* community structure among depths, however few clades were restricted to depth.

A few *Symbiodinium* clades were only recorded in shallow environments (D3 variant, C15n, C15p,o, A1c, C65 variant2) and others exclusively from deep sites (C170a, A1 variant, C1) (Fig. 3-2) however, analysis of similarity (ANOSIM) showed that *Symbiodinium* assemblage overall did not change significantly between the two depths and variability was only 1% (ANOSIM, R=0.01) revealing that the *Symbiodinium* assemblage was stable between the two depth levels. Similarly, the *Symbiodinium* community structure did not change across locations sampled (ANOSIM, $p>0.05$, $R = -.0008$) and therefore did not seem to vary within a species at sites with different thermal conditions (Table 3-1).

Unlike the stability of *Symbiodinium* across depths and sites, *Symbiodinium* community was highly variable (64% variability) among coral species (ANOSIM, $p<0.001$, $R=0.64$), where each coral host harbored different clade types revealing a high degree of symbiont-host specificity (Fig 3-3). Each coral host harbored one or multiple symbiont types that was specific to the host across sites and depths. Specifically, *P. nodifera* mainly harbored C15 (80 and 100% for shallow and deep samples respectively), however shallow samples contained C15p,o (13%) and C15n (7%) clade types which were not recorded at deep samples (Fig 3-3). Similarly, *S. trocheliophorum* harbored only C65 variant 1 types that contributed ca.92 and 100% to shallow and deep samples respectively across surveyed sites (Fig 3-3, Table 3-1), and *X. umbellate* was specific to a new recorded subclade C171 type (82 and 92% for shallow and deep samples respectively).

However, *P. damicornis* mainly harbored *Symbiodinium* clade A1 and A1c in shallow samples (86% combined), but deep samples had different assemblages and harbored C1h (80%) instead of A1 clade. *F. favus* harbored four clade types (i.e. C3z, C1 variant, C1 and C41), mainly C3z (50%) and C1 variant (40%) in shallow samples, which changed to 62%

C1 variant and 23% C3z clade at deep samples (Fig 3-3). Similarly, *S. hystrix* harbored clade C170 (80% and 60% for shallow and deep samples respectively), while deep samples harbored clade C170a type (20%) however, it shared A1 clade type (20% in both depths) as in *P. damicornis* (Fig 3-3, Table 3-1). Interestingly, shallow *S. hystrix* was the only coral that had different symbiont clade types over sites to A1 clade in shallow and C170a type in deep samples in southern most site (Meritte and Wadi El Gemal respectively) (see Table 3-1).

The overall pattern of *Symbiodinium* distribution was stable across locations despite the different thermal histories. Conversely, *Symbiodinium* community was highly specific to its coral hosts, and only a few hosts shared its symbiont type with others (e.g. A1 in both *P. damicornis* and *S. hystrix*). Moreover, depth did not significantly affect symbionts community although some differences in cladal dominance was apparent in some hosts (e.g. *P. damicornis* and *F. favus*).

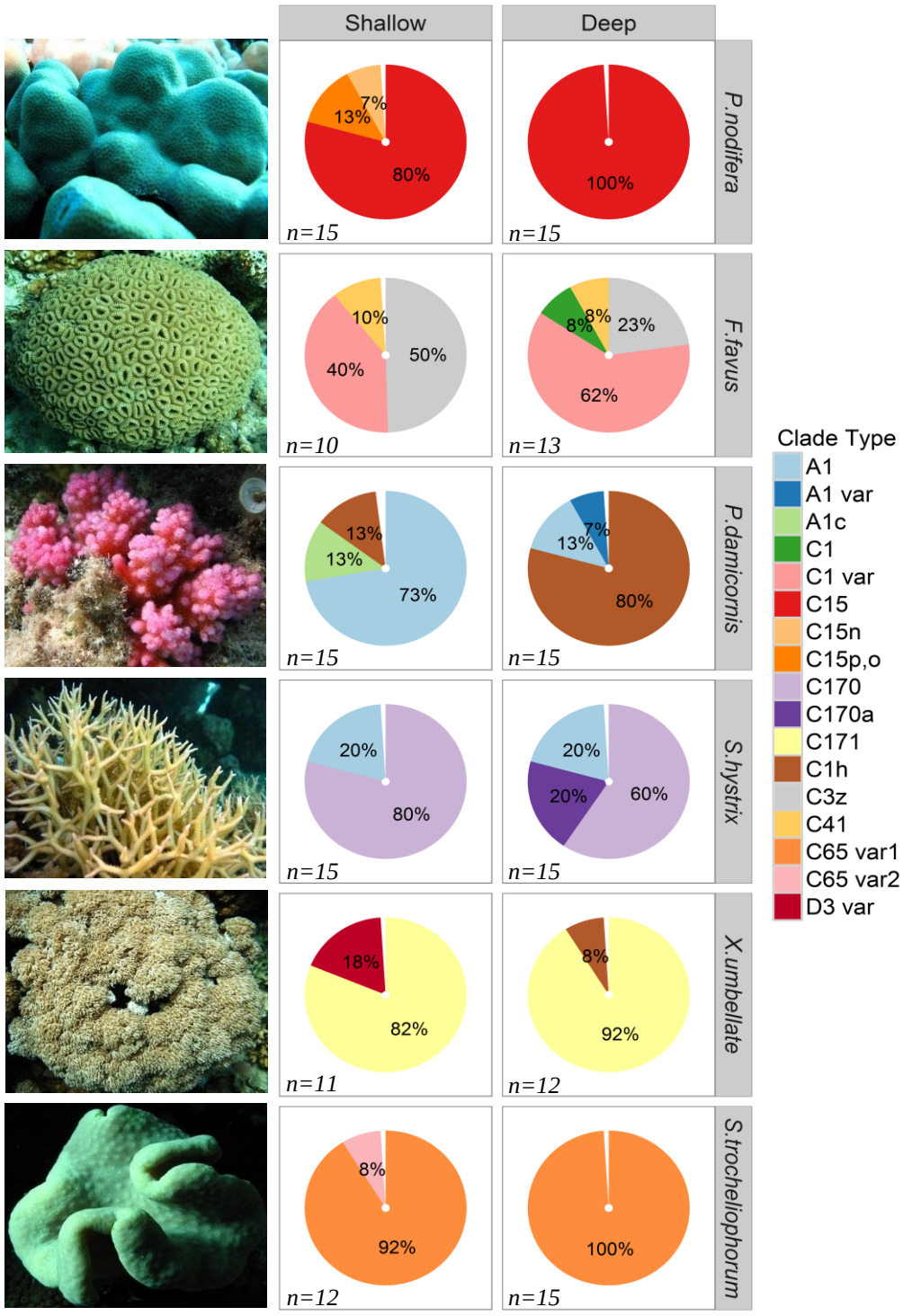

Figure **3-3**. Percentage of *Symbiodinium* clade ITS2-types of six coral species at both depths across all sampling sites. Plot represense each coral species sepertalty accors sites (i.e. symbionts pooled for each corals species across sites) at two depth levels and demonstrating high host-sybiont spesificty and limited effect of depth. Samples were collected during Febreury/March, 2013 form five sites along Egyptian Red Sea coast (total n=163).

Table **3-1** *Symbiodinium* clade ITS2-types of collected samples from differnt coral species at two depths. Identification carried out by ITS2 PCR-DGGE fingerprint profile. Sites are arranged in order from north (low temperature) to south (high temperature) and SST indicated by color scale arow.

Species/Sites	***Porites nodifera***		***Favia favus***		***Pocillopora damicornis***		***Seriatopora hystrix***		***Xenia umbellate***		***Sarcophyton trocheliophorum***	
	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep
Abo Galloum	C15	C15	NA	C1	A1c	C1h	C170	C170	C171	C171	C65	C65 var
	C15o,p	C15	C3z	C3z	A1	C1h	C170	C170	C171	C171	C65 var	C65 var
	C15	C15	NANA	C41	C1h	C1h	C170	C170	C171	C171	C65 var	C65 var
Ras Mohamed	C15	C15	NA	C1	A1	C1h	C170	C170	NA	C171	C65 var	C65 var
	C15	C15	NA	C1 var	A1	A1	C170	C170	D3 var	C171	C65 var	C65 var
	C15	C15	NA	C1 var	A1	A1	C170	C170	D3 var	C171	NA	C65 var
Abo Galawa	C15	C15	C1 var	C1 var	A1	C1h	C170	C170	C171	NA	C65 var	C65 var
	C15n	C15	C3z	NA	A1	C1h	C170	C170	C171	NA	C65 var	C65 var
	C15	C15	C41	C1 var	A1	C1h	C170	C170	C171	NA	C65 var	C65 var
Meritte	C15	C15	C1 var	C3z	A1c	C1h	C170	A1	C171	C171	C65 var	C65 var
	C15o,p	C15	C3z	C1 var	A1	C1h	C170	A1	C171	C171	C65 var	C65 var
	C15	C15	C3z	C1 var	C1h	C1h	A1	A1	NA	C171		C65 var
Wadi El Gemal	C15	C15	C1 var	C3z	A1	A1 var1	A1	C170a	C171	C171	C65 var	C65 var
	C15	C15	C3z	C1 var	A1	C1h	A1	C170a	NA	C1h	C65 var	C65 var
	C15	C15	C1 var	NA	A1	C1h	C170	C170a	NA	C171	NA	C65 var
No. samples=	15	15	10	13	15	15	15	15	11	12	12	15
No. Clade=	3	1	3	4	3	3	2	3	2	2	2	1

*NA is not available clade ID due to missing samples replicates, PCR amplification inhibition or low quality DGGE sequence

3.4.2. Bacterial Community – 16S Metagenomics analysis:

Taxonomic profile __ Sequencing of bacterial 16S library associated with six coral species and water samples at five surveyed sites produced in total 23.7m reads that ranged from 46.3k to 3.3m reads per sample (median=128.3k reads). Overall microbial community composed of 11161 OTUs belonging to 56 phyla. Taxonomy profile was highly conserved among sites, depth levels and coral species, where Proteobacteria was the predominant phylum and composed >80% of total community, followed by Bacteriodetes and Cyanobacteria (Fig. 3-4). Data revealed that only 11 OTUs composed ca.52% of the total bacterial community abundance (Table 3-2), and remaining OTUs (n=11150) shaped the remaining microbial community structure (48%) without defined dominated taxa (i.e. their relative abundance was <1% of total abundance each). Analysis of Proteobacteria revealed that it was composed mainly of α and γ Proteobacteria (ca.70% combined); where specifically, both *Alteromonas* sp. and *Pseudoalteromonas* spp. (that were both belonging to γ Proteobacteria) made up ca. 38% of the total microbial abundance (ca. 25% and 13% respectively) (Fig. 3-4, Table 3-2).

Table **3-2**. Taxonomic profile of predominant OTUs and its relative abundance (%) in total microbial community. Only 11 OTUs composed ca.52% of total microbial abundance of all coral species across sites

Phylum	Class	Order	Family	genus	%
Proteobacteria	γ proteobacteria	Alteromonadales	Alteromonadaceae	*Alteromonas*	25
Proteobacteria	γ proteobacteria	Vibrionales	Pseudoalteromonadacea	*Pseudoalteromonas*	9
Proteobacteria	γ proteobacteria	Vibrionales	Pseudoalteromonadacea	*Pseudoalteromonas*	4
Proteobacteria	α proteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	3
Proteobacteria	γ proteobacteria	Oceanospirillales	Endozoicimonaceae	*NA*	2
Proteobacteria	γ proteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	2
Proteobacteria	γ proteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	2
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*NA*	1.8
Proteobacteria	γ proteobacteria	Vibrionales	Vibrionaceae	*Vibrio fortis*	1.6
Proteobacteria	α proteobacteria	Rhodobacterales	Rhodobacteraceae	*Loktanella*	1.3
Proteobacteria	α proteobacteria	Sphingomonadales	Erythrobacteraceae	*Erythrobacter*	1.1

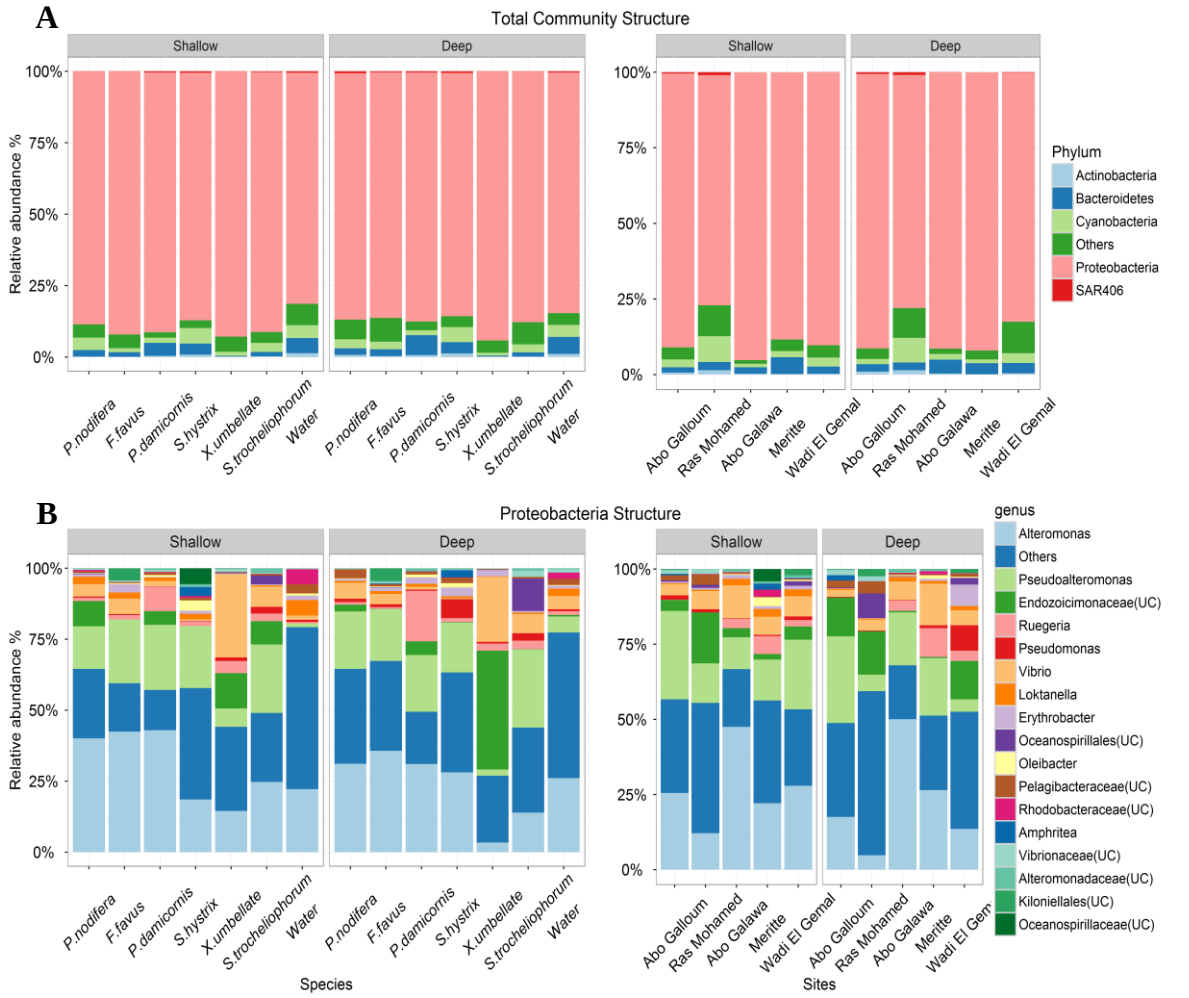

Figure **3-4**. Microbial community structure associated with coral species (include water) along surveyed sites on Egyptian Red Sea coast. Taxonomy profile of total microbial community structure at phylum level (A) showed the predominance of phylum was Proteobacteria in among sites and coral species in both depths. Analysis of Proteobacteria into genus level (B) reveled dominance of *Alteromonas* and *Pseudoaltheromonas* (belonging to γ Proteobacteria) and composed combined ca.48% of total population. Due to high number of taxa, all taxa <0.5% of relative abundance were assigned into 'others' category for clarity, and the unclassified taxa to genus level denoted by (UC).

Microbial diversity analysis __Chao1 richness estimator, inverse Simpson and Shannon diversity indices were performed on OUT table (i.e. samples) to assess the microbial diversity of each coral sample at all sites (n=168), then multifactorial ANOVA was performed on the outcome. Diversity of microbial community of water samples was stable across sites and depth (Table 3-3), but OTUs richness (i.e. Choa1) did differ and slightly changed among depth (ANOVA, Df=1, F=6.4, $p<0.05$).

Similar to water samples, the diversity of microbial community generally was not changed significantly at the two depths, but the interaction between depth and corals species or sites was statistically influencing the microbial diversity (Table 3-3). Conversely, microbial diversity varied significantly between sites and coral species and their interactions (ANOVA, $p<0.05$ for all diversity indices, see Table 3-3).

Table **3-3**. Summary statistics of ANOVA performed on diversity indices outcome to test the difference of microbial community among coral species and water samples at all surveyed sites and depths.

	Factor/ diversity		**Choa1 estimator**		**Inverse Simpson**		**Shannon**	
		Df	F value	P value	F value	P value	F value	P value
Corals	Depth	1	2.4	p>0.05	1.5	p>0.05	3.8	p>0.5
	Sites	4	17.8	p<0.001***	12.4	p<0.001***	17	p<0.001***
	Coral Species	5	8.2	p<0.001***	3.4	p<0.01**	5.7	p<0.001***
	Depth*Sites	4	5.3	p<0.001***	3.7	p<0.01**	5.5	p>0.001***
	Depth*Species	5	4.5	p<0.01**	2.8	p<0.05*	3.9	p<0.01**
	Sites*Species	20	2.4	p<0.01**	4	p<0.001***	4	p<0.001***
	Depth*Sites*Species	16	2	p<0.05*	1.9	p>0.05	2.1	p<0.05*
Water	Depth	1	6.4	p<0.05*	0.06	p>0.05	0.4	p>0.05
	Sites	4	1.9	p>0.05	1.1	p>0.05	2.5	p>0.05
	Depth*Sites	4	1.1	p>0.05	0.6	p>0.05	0.6	p>0.05

To investigate the dispersion of microbial community structure among factors, Principle Coordinate Analysis (PCoA) was performed on OTU data for all samples (n=168) based on Bray-Curtis dissimilarity distance. Overall, microbial community structure was highly shared among factors where no defined separation was observed among sites or coral species in both depths (Fig. 3-5). Despite this, statistical analysis confirmed that microbial community structure was heterogeneous and differed significantly between sites and coral species (PERMANOVA, p<0,01, see Table 3-4), where both sites and corals explained 18% and 14% of the microbial community variation respectively (PERMANONVA, r^2= 0.18 and 0.14 for sites and species respectively, see (Table 3-4). Similarly, microbial community structure changed significantly between shallow and deep samples (PERMANOVA, p<0.01), although the explained variation by depth was only ca. 1% for coral samples and ca.

5% between water samples (PERMANOVA, r^2=0.01 and 0.05 for coral and water respectively, see Table 3-4). This pattern was further confirmed by analysis of similarity statistical test (ANOSIM, see Table 3-4).

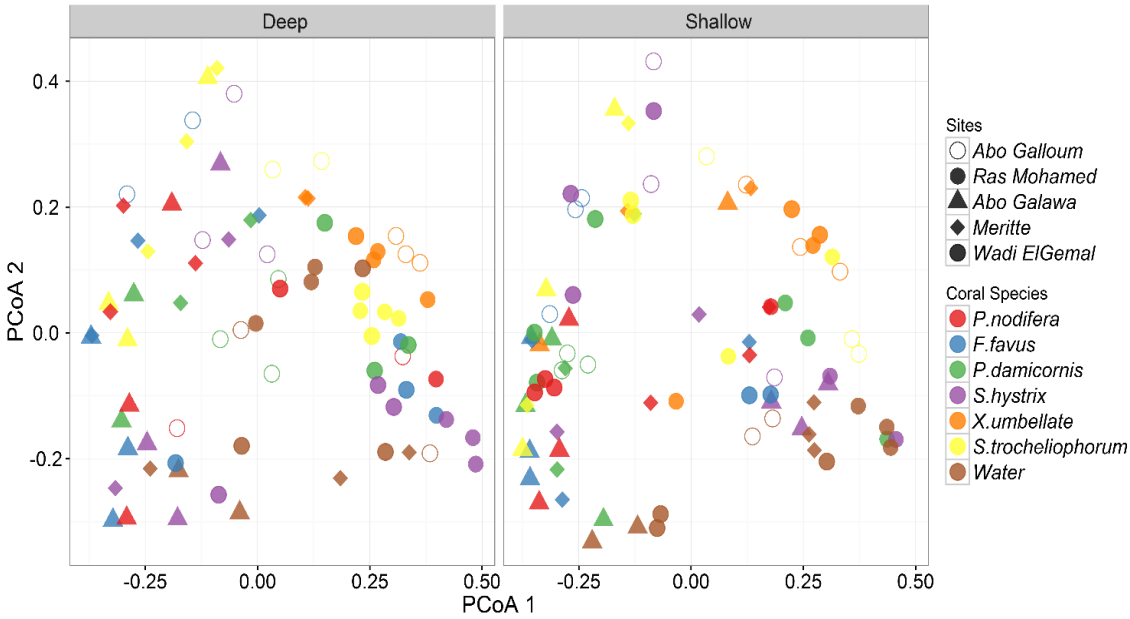

Figure **3-5**. Principle Coordinated Analysis plot (PCoA) based on Bray-Curtis dissimilarity matrix of microbial community sampled from different coral species at different sites and depth along the Egyptian Red Sea. The PCoA plot that shows sites (symbols) and coral species (colors) demonstration little differences of microbial community among depths, as well as the separation between coral species or sites was undefined pattern, except water samples that showed very slight separation from coral species

Table **3-4**. Statistical summary of permutation multivariate analysis of variance (PERMANOVA) and analysis of similarity (ANOSIM) that performed on microbial community associated with six coral species collected from five sites at two depth levels based on Bray-Curtis dissimilarity matrix. Multifactorial analysis (PERMAONVA) performed to investigate the influence of site, depth, coral species and their interactions on microbial community composition and used permutation level 999. Both analysis was performed by *adonis* and *anosim* functions in "R" ('vegan' package) with statistical significance level <0.05.

Factor/ Analysis		**PERMANOVA analysis**						**ANOSIM**	
		Df	Sum of	Mean	F Model	R^2	P value	R value	P value
Coral Species	Depth	1	0.442	0.442	2.8	0.011	0.01**	0.016	0.048*
	Site	4	7.778	1.944	12.4	0.186	0.01**	0.304	0.001**
	Coral Species	5	5.888	1.178	7.5	0.141	0.01**	0.199	0.001**
	Depth*Site	4	1.942	0.486	3.1	0.046	0.01**	-------	-------
	Depth* Coral species	5	1.156	0.231	1.4	0.028	0.01**	-------	-------
	Sites* Coral species	20	8.038	0.402	2.5	0.192	0.01**	-------	-------
	Depth*Site*Coral	16	3.442	0.215	1.4	0.082	0.01**	-------	-------
Water	Depth	1	0.364	0.364	0.036	0.054	0.01**	0.032	0.166
	Sites	4	2.272	0.568	0.56	0.337	0.01**	0.304	0.001**
	Depth*Sites	4	1.329	0.332	0.33	0.197	0.01**	-------	-------

To further investigate this pattern, the change of microbial community between coral species within each site (i.e. data was split to separate sites, but include all coral species) was tested, as well as the change across sites for each coral species (i.e. each coral species at all sites). Data confirmed the later pattern and the structure of microbial community varied across sites for each coral species, as well as between different coral species within the same site (PERMANOVA, $p<0.001$). Depth was not generally statistically different in either case except in some coral species (i.e. *P. damicornis* and *X. umbellate*) and only one site (Wadi El Gemal) that showed difference in microbial community structure among depths (Table S2 – Appendix).

Overall, the microbial community associated with six coral species inhabited five sites at two depth levels appeared statistically different among sites and coral species (i.e. PERMAOVA and ANOSIM), but not much among depths. This highlights that the microbial community was not homogenous across sites or among coral species which reflect a degree of microbial specificity to both sites and coral species.

*Association analysis*__ To determine the components of the microbial community that caused variation among sites and coral species, the specificity of the microbial community for each site and coral species was examined using indicator species analysis (Cáceres & Legendre, 2009). This test returns a list of microbial species (i.e. OTUs) significantly associated ($p<0.05$) with the key factor (i.e. sites, coral species). Out of the total 11161 OTUs representing the entire microbial community, 3515 OTUs (ca. 32%) were significantly associated with sites, whilst ca.68% of the microbial community was shared between sites (Fig. 3-6A). Ras Mohamed had the highest number of associated indicator species (ca.14%, 1517 OTUs) followed by Meritte (ca. 8%, 851 OTU) and Wadi El Gemal

(ca. 7%, 746 OTUs) (Fig. 3-6A). The contribution of indicator species to total microbial community abundance varied between sites ranging from ca.10% (ca. 411 k reads) at Wadi El Gemal to 62% (ca. 2 m reads) at Ras Mohamed (Fig. 3-6B).

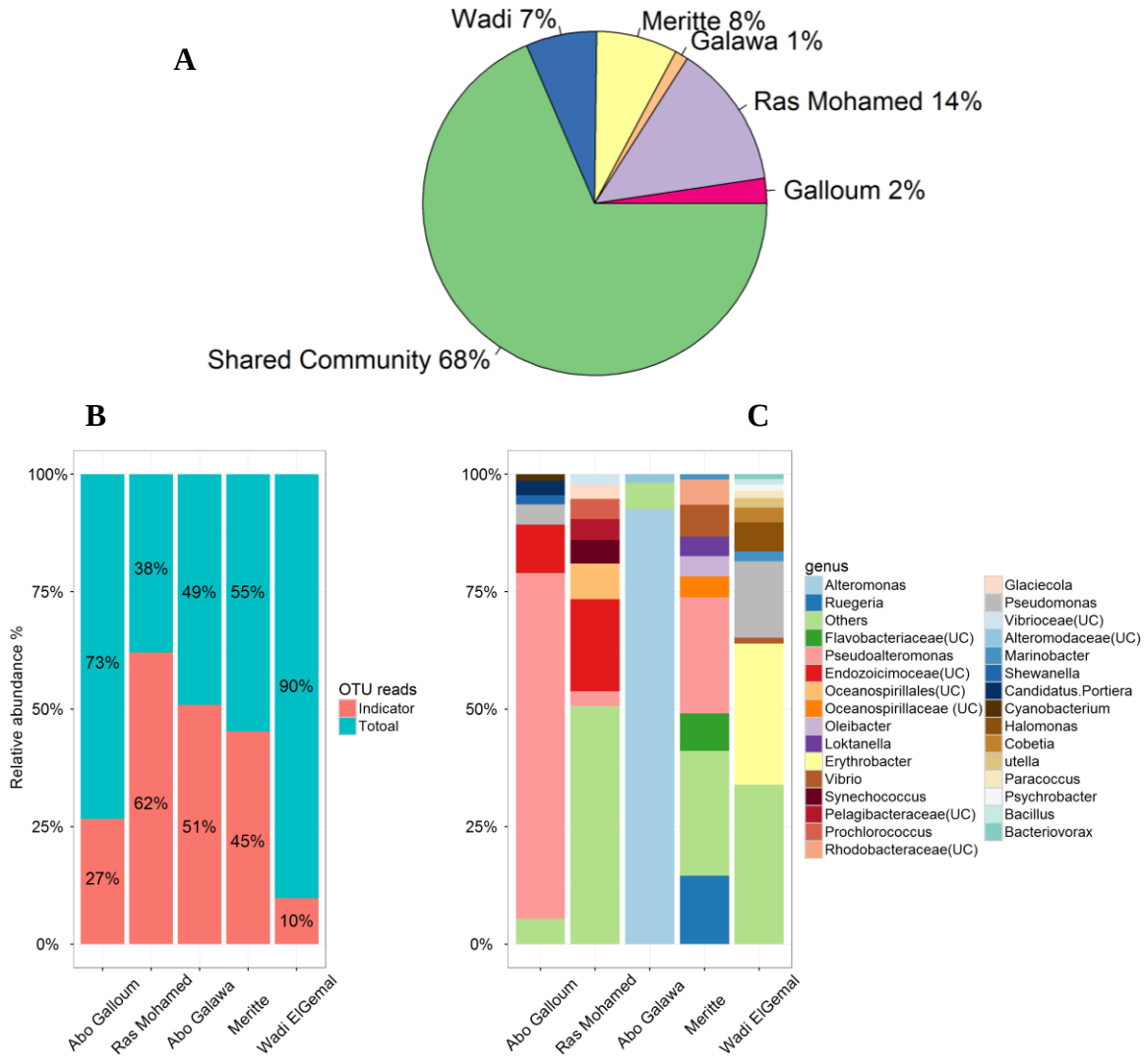

Figure **3-4**. Panel plot of indicator species of microbial community that was significantly ($p<0.05$) associated with each sampling sites using Indi-species package in 'R'. Pie chart (A) represents the percentage of OTUs that was associated with each site, while the contribution of those OTUs in total relative abundance at each site represented in barplot (B). The composition of indicator species and its relative abundance represented in barplot (C) at genus level where all taxa below 2% of total relative abundance was assigned to others and unclassified genus was denoted UC.

The relative abundance within indicator species at each sites was different, and each site was dominated by different taxa (Fig. 3-6C). Specifically, Abo Galloum was dominated by *Pseudoalteromos* spp. (ca.69% of total indicator species), while *Alteromonas* spp. was dominant at Abo Galawa (ca.91%), and both *Pseudoalteromos* spp. (ca.25%), *Ruegeria* spp. (ca. 14%) and unclassified Flavobacteriaceae family (ca. 8%) were highly abundant in Meritte (Fig. 3-6C). Interestingly, Wadi El Gemal (the warmest site) was dominated by *Erythrobacter* sp. (ca.30%).

Similarly, the number of OTUs that were significantly associated with coral species and water samples combined composed ca.33% of total microbial OTUs community, half of them were specific only for water samples (1808 OTUs, ca.16%), and 16.6% (1851 OTUs) was associated with coral species (Fig. 3-7A). Their contribution in total relative abundance of total microbial community abundance varied among coral species ranging from 6% for *F. favus* to 64% in *X. umbellate*, while it composed 49% of total abundance of microbial community in water samples (Fig. 3-7B).

The composition of relative abundance of indicator species differed between coral species; specifically, *P. nodifera* and *F. favus*, both of which were dominated by Endozoicimonaceae (ca.49% - unclassified family) and Kiloniellales (ca.52% - unclassified Order) respectively (Fig. 3-7C). Similarly, *P. damicornis* was associated with *Ruegeria* sp. (ca.63%) and Endozoicimonaceae (ca.30%), while *X. umbellate* was dominated by Endozoicimonaceae (ca.46%) and *Vibrio* spp. (ca.40.5%). Remaining coral species were associated mainly with Order Oceanospirillales (ca.41.4% and 34.4% for *S. trocheliophorum* and *S. hystrix* respectively), while water samples was not dominated by defined microbial taxa (Rhodobacteraceae, ca.7.5%, was the dominant Family) (Fig. 3-7C).

This data highlighted that most corals were associated significantly with the Order Oceanospirillales (Endozoicimonaceae is a Family member), unlike those specific to sites that were belonging to different functional taxa (Table 3-5) which indicates that site-specific microbes perform different function across sites.

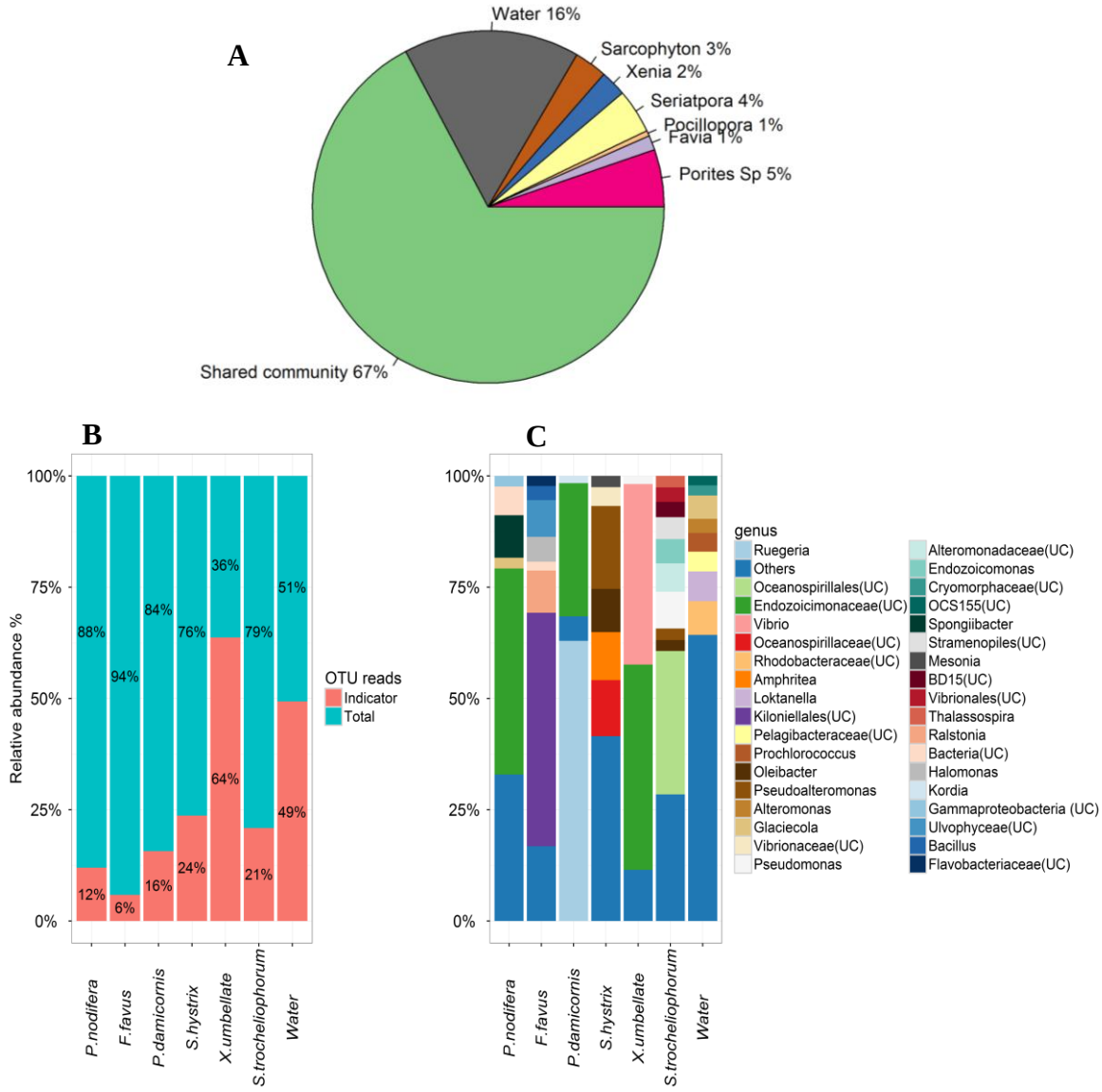

Figure **3-5**. Panel plot of indicator species of microbial community that was significantly ($p<0.05$) associated with each coral species include water samples using Indi-species package in 'R'. Pie chart (A) represent the percentage of OTUs that was associated with each coral species, while the contribution of those OTUs in total relative abundance within each coral microbial community represented in barplot (B). The composition of indicator species and its relative abundance represented in barplot (C) at genus level, while all taxa <1% of relative abundance were assigned to "others" and unclassified txa to genus level denoted UC.

Table **3-5**. Summary of taxonomic and functional profile of dominant/abundant bacteria (Indicator Species) associated significantly with sites along Egyptian Red Sea coast.

Sites	OTUs	Taxa	No. of reads	Relative %	Function	Reference
Abo Galloum	OTU128489size=12 OTU123size=14364 OTU43150size=38 OTU1781size=1005 OTU181782size=8 OTU79364size=20 OTU2561size=655	*Pseudoalteromonas sp*	794730	69	It's mainly pigmented bacteria with photosynthetic activity that produce biologically active molecules that are beneficial to eukaryotes. In addition to production biofilm, antibiotics, antifouling compounds. Also induce coral settlement.	(Holmström *et al.*, 1999; Webster *et al.*, 2004; Dobretsov *et al.*, 2006; Bowman, 2007)
	OTU28size=47776	Endozoicimoceae	108700	9.5	Endosymbiot that recognize, communicate and modulate its coral host. Also it responsible for and sulfur cycle and antimicrobial production.	(Raina *et al.*, 2009; Rua *et al.*, 2014; Ding *et al.*, 2016)
Ras Mohamed	OTU18size=65034 OTU29size=46392	Endozoicimoceae	407125	20		
	OTU12size=97275	Oceanospirillales	158076	7.6	Endosymbiont has ability to degrade sulfur compounds (i.e. Dimethyl-sulfoniopropionate), hydrocarbons and amino acids. It usually found with healthy corals.	(Jensen *et al.*, 2010; Raina *et al.*, 2010; Cardenas *et al.*, 2012; Mason *et al.*, 2012)
Abo Galawa	OTU1size=1901745	*Alteromonas sp*	2310182	91.3	Copiotrophic (i.e. survive in rich nutrients environments) and pigmented bacteria that has ability to produce melanin for UV irradiation protection, degrade sulfur and nitrogen compound, and produce antibiotics.	(Gauthier *et al.*, 1975; Ivanova *et al.*, 1996; Raina *et al.*, 2009)
Meritte	OTU2size=650492 OTU45size=35108 OTU173size=8687	*Pseudoalteromonas sp*	781467	25		
	OTU4size=345091	*Ruegeria sp*	446316	14.3	Basically found with unhealthy corals and has role in horizontal gene transfer, which may help hosts and microbial associates adapt to environmental challenges in short time periods	(McDaniel *et al.*, 2010, 2012; Casey *et al.*, 2015)
Wadi El Gemal	OTU11size=101880	*Erythrobacter sp*	123557	30.0	It's phototropic bacteria that has bacteriochlorophyll-a (B-Chl *a*) and large amount of carotenoids	(Yurkov *et al.*, 1994; Denner *et al.*, 2002; Koblízek *et al.*, 2003)
	OTU62size=28194	*Pseudomonas sp*	66949	16.3	It's antimicrobial resistant bacteria	(ElAhwany *et al.*, 2015)
	OTU208size=7085	*Halomonas sp*	13771	3.3	It's traditionally extreme halophiles	(Lee *et al.*, 2005)

3.5. Discussion

The acclimation capacity of corals to thermal stress is a key factor that determine their potential to survive in the future (Bellantuono *et al.*, 2012b) however, understanding the role of the holobiont in driving thermal tolerance is not well resolved. Most attempts to understand coral acclimation mechanisms to thermal stress have mainly focused on the physiology of the various genetic variants of *Symbiodinium* or to a lesser extent, the microbial community in isolation (e.g. Oppen *et al.*, 2009; Hume *et al.*, 2013; Jessen *et al.*, 2013; Casey *et al.*, 2015b). However, it is most likely that a combination of both the zooxanthellae and other microbes (i.e. the microbiome) drive coral health and its ability to cope with changing environmental conditions including acute and chronic stress events. In the current study, the composition of all microbiome components was studied across different latitudes of the Egyptian Red Sea coast, an area that can be considered 'extreme' when compared to other reef sites around the world.

The results showed high level of host-symbiont (zooxanthellae) specificity across latitudes which was consistent with previous studies (van Oppen *et al.*, 2001; LaJeunesse *et al.*, 2003, 2004, 2010; Frade *et al.*, 2008). Tonk *et al* (2013) found that the host species identity plays a major role in *Symbiodinium* distribution on the Great Barrier Reef, and shaped the overall *Symbiodinium* distribution and diversity. This relationship may be attributed to host driven factors that influence micro-habitat conditions for the symbiont such as, colony morphology and tissue thickness which influence light absorption (Enríquez *et al.*, 2005), as well as availability of host pigments which facilitate photosynthesis (Dove *et al.*, 2008).

Importantly, symbiont acquisition strategy (i.e. larval symbionts passed on from parental generation – "vertical" transmission, or symbiont taken up direct from the external

environment – "horizontal" transmission) (Stat *et al.*, 2008a) can drive host-symbiont specificity and how it varies over time. Shlesinger *et al* (1998) reported that 87.5% of reef-building corals in the northern Red Sea are broadcasting spawners (i.e. producing gametes), and therefore, must acquire their symbiont from the environmental pool (Richmond & Hunter, 1990). Barneah *et al* (2004) found that all hosts collected from the Red Sea using horizontal transmission harbored symbionts belonging to C clade, while those with vertical transmission uniquely harbored symbionts from clade A. This finding supports our results that showed prevalence of clade C types (ca.85%) across sites and coral species, and also consistent with previous studies in the Red Sea (Baker *et al.*, 2005) and Indo-Pacific region (LaJeunesse *et al.*, 2003; Wicks *et al.*, 2010; Lien *et al.*, 2012; Yang *et al.*, 2012). Stat *et al* (2008b) found that clade C is beneficial to coral host by fixing more carbon than clade A and hence, provide sufficient nutrients to coral host. Therefore, during symbiont acquisition in horizontal transmission, larvae has the freedom to choose the beneficial clade according to i) its availability (Buddemeier & Fautin, 1993; Douglas, 1998) and ii) surrounding environmental stressors during the establishment of symbiosis (Abrego *et al.*, 2012). The limitation of clade A to hosts with vertical transmission suggests a co-evolution of the host-symbiont relationship, while clade C symbionts that characterized by large sub-clade variability exhibit plasticity of physiological capabilities (Barneah *et al.*, 2004).

The results also showed that *Symbiodinium* ITS2-type community did not change over sites despite increases of SST southward as hypothesized. This may reveal high plasticity of clade's physiology of symbionts therefore, the genetic differences between clade types are not the main driver for thermal tolerance as suggested by Tonk *et al* (2013). Ziegler *et al* (2014) found change in symbiont cell density and photochemical pigments between

different light regimes in the Red Sea. Also, Sawall *et al* (2015) found extensive phenotypic plasticity of clade A1 hosted by *Pocillopora verrucosa* along latitudinal gradients of the Red Sea accompanied with change in metabolic and photosynthetic rate. Similarly, Hoadley *et al* (2015) reported change of cellular volume, protein, and lipid content of symbionts as a response to heat stress that correlated to increase their thermal tolerance (see also, Hoadley *et al.*, 2016). Tchernov *et al.*, (2004) found that the lipid composition of thylakoid membranes of *Symbiodinium* spp. is the key driver in determining its thermal susceptibility within the same symbiont clade type. This highlights that the different phenotypic capability of symbionts is an important factor can determine the thermal tolerance of corals (see chapter 5; see also; Howells *et al.*, 2016; Levin *et al.*, 2016).

In contrast to *Symbiodinium*, other components of the microbiome (i.e. microbial community) were extremely diverse (i.e. 11161 OTUs) as previously reported elsewhere (Rohwer & Kelley, 2004; Bourne & Munn, 2005; Bayer *et al.*, 2013a), although growing evidence suggests that coral species contain specific and ubiquitous microbial community (Rohwer & Kelley, 2004; Ainsworth *et al.*, 2015). Hernandez-Agreda *et al* (2016) proposed that the microbial community should be categorized into three components ; 1) The ubiquitous core microbiome (i.e. ubiquitous microbiome of very few symbiotic bacteria with coral host facilitate the success of *Symbiodinium* endosymbiosis – e.g. *Ralstonia* sp*,* see also Ainsworth *et al* 2015), 2) The spatial and local/regional microbial community (i.e. core microbial community that filling functional niche and driven by local condition <100 phylotypes), and 3) The highly variable microbial community (i.e. that rapidly respond to changes in local environmental and biological conditions across spatial and temporal scale - >100 phylotypes). This categorization defines the observed variations of microbial

community among corals across latitudes in this study, particularly those specific to sites that were taxonomically/functionally different.

The results showed that ca.15% of OTUs were specific for coral species while ca.30% were associated with sites. Ritchie & Smith (2004) suggested that microbial communities are likely specific to coral species, and Hong *et al* (2009) found high species specificity in the bacterial assemblage associated with corals from the Caribbean Sea. The species-specific bacteria were further discovered not only with corals (Rohwer *et al.*, 2002; Reis *et al.*, 2009; de Castro *et al.*, 2010; Kvennefors *et al.*, 2012; Carlos *et al.*, 2013), but also in other marine organisms (e.g. Fieseler *et al.*, 2004; Fraune & Bosch, 2007; Di Camillo *et al.*, 2012). This pattern confirmed presence of ubiquities and conspecific phylotypes that fill a functional niche that fit local environmental conditions as proposed by Hernandez-Agreda *et al* (2016). For example and consistent with our results, Bayer *et al* (2013) reported the dominance of Endozoicimoceae (Order Oceanospirillales) in *S. pistillata* inhabiting the Red Sea and appear to have an intimate relationship with the coral. Similarly, Ainsworth *et al* (2015) reported two ubiquities bacterial phylotypes (*Ralstonia* sp and *Actinobacter* sp) that specific to corals worldwide and facilitate the symbiotic relationship within different environmental regimes. This highlights our need to identify the core microbiome associated with corals and examine how it play a role in holobiont's function to resist environmental stressors (see chapter 5).

Consistently, the spatial distribution of the microbial community between sites can be explained by localized environmental variability leading to site-specific microbial community. Ziegler *et al* (2015) reported significant differences in microbial communities associated with corals inhabiting impacted sites by anthropogenic activity in Jeddah, Saudi suggesting that particular microbial taxa can be indictors for anthropogenic footprint,

regardless of healthy appearance of corals. Similarly, high variation in microbial community was reported among sites in response to salinity and depth at Thuwal, Red Sea as reported by Lee *et al* (2012b). The results support previous findings that site does play a significant role in influencing the community structure of the microbiome. Despite that the functional role of these microbes still remains untested, but clearly both coral species, the local environmental setting and the interaction between coral species and the environment influence the microbiome of a coral host, therefore coral fitness. For example, Röthig *et al* (2016) reported a shift in microbial community that performed different functional role as a response to high salinity.

However, the microbial community was statistically different among sites and corals species, the ordination plot (PCoA) did not show distinct dispersion of particular factor (i.e. sites, corals and depth). This may be attributed to dominance of eleven OTUs across sites and species and composed ca.52% (Table.2) of total relative abundance therefore, data was masked by the dominance of few OTUs that composed the majority of the community. The dominance of the 11 OTUs may be a result of the relatively stable temperatures experienced during the sampling period (February/March, 2013) across all sites from 21-22.1 °C across sites (long term mean of winter SST, 2003-2014 based on MODIS data), and for a fuller picture the annual variability of microbial communities within coral species and across sites should be determined. Lee *et al* (2015) recorded a significant shift of microbial community associated with *Acropora muricata* only after exposure to 3°C increase in water temperature demonstrating that microbial community change under certain conditions. The community appears conserved under a set of environmental conditions for any given species within a site but changes in environmental conditions especially conditions considered stressful is

associated with shifts in the microbiome (see Bourne *et al.*, 2008; Webster *et al.*, 2011). This finding is consistent with previous studies that did not report significant changes of microbial communities among seasons when temperature did not exceed the maximum summer temperature (e.g. Carlos *et al.*, 2013).

One interesting outcome of this study revealed significant association of *Erythrobacter* spp. with warmest southernmost site (i.e. Wadi El Gemal), where its abundance increased six fold over latitudinal gradients (i.e. 450 to 3650 reads for Abo Galloum to Wadi El Gemal respectively- see Figure S10 - Appendix). This group of bacteria is rarely reported in corals in high abundance (Nelson *et al.*, 2013), but Ceh *et al* (2013) reported high abundance of *Erythrobacter* sp. during reproduction of *Acropora tenuis* in GBR. Also, Lai (2012) identified novel species, *Erythrobacter pelagi* from the Red Sea that was able to survive across a 10-35°C temperature range. This bacteria group is characterized by a presence of bacterial chlorophyll-a and large amounts of carotenoid (Yurkov *et al.*, 1994; Denner *et al.*, 2002; Koblízek *et al.*, 2003; Yuki *et al.*, 2012).

Kelly *et al* (2014) found reefs located near the equator were dominated by microbiomes containing more encoding chlorophyll-*a* biosynthesis and photosystem I/II bacteria suggesting that holobiont core microbiome is locally adapted. Also, Lee *et al* (2015) investigated the microbial community dynamics associated with *Acropora muricata* during serial thermal stress experiment (26-33°C), found that Sphingobacteria (i.e. higher taxa of *Erythrobacter* spp) became one of the dominant bacterial groups after being exposed to extreme thermal stress (i.e. 33 °C). I argue that this bacterial group may support coral host by supplementing photosynthetic activity (via B-Chl*a*) and antioxidant activities (via

carotenoid) to increase the holobiont fitness during heat stress, but further investigations are needed to test its functional role in coral health.

To conclude, this work exhibited conserved *Symbiodinium* community, but high variable microbial community across different sites. This highlights that *Symbiodinium* spp. may have broad thermal niche (i.e. phenotypic plasticity) in this region enabling them to populate broad geographical and environmental ranges (Sampayo *et al.*, 2008; Sawall *et al.*, 2015). In contrast, the non-zooxanthellate microbiome (i.e. microbial community) varied significantly across sites, and therefore demonstrated a great degree of plasticity. The results demonstrate a link between microbial plasticity and broad environmental niche; however, it is not clear how it enables these corals actually responds (tolerate) to stressors. Therefore, the next chapter investigates the response of microbiome under thermal stress at different thermal regime to provide an insight of its role in holobiont fitness.

Chapter 4

4. *The role of the microbiome in the acclimation of corals inhabiting different thermal regimes*

4.1. Abstract

Coral reef ecosystems are highly sensitive to thermal stress, however, some populations exhibit high thermal resistance. The acclimation mechanisms of corals living in natural extreme environments have been a major focus of recent research because it is critical to understanding how corals can survive the increasing trend of global warming. The spatial variability of the Red Sea water temperature and particularly thermal anomalies, makes it a perfect model system to examine the acclimation mechanisms of reef-building corals. Herein, the acclimation of corals inhabiting different thermal regimes within the Red Sea was experimentally investigated (i.e. Hurghada and Jeddah) to identify regional acclimation patterns and the role of the microbiome (i.e. symbiont type and microbial community) in corals thermal tolerance. The photochemical efficiency (i.e. *r*ETR and F_v/F_m) of corals exposed to +3°C above local summer mean declined at Jeddah in comparison to Hurghada which remained healthy under this thermal stress. This difference was not explained by symbiont clade types which did not differ within host type at the sites. Conversely, heat treatment did not influence the microbial diversity at Hurghada, but there was shift in the microbial diversity of corals at Jeddah as a response to heat stress. Also, the decline in photochemical efficiency in Jeddah (e.g. in *P. damicornis*) associated with shift from dominance of Endozoicimonaceae to cyanobacteria. Interestingly, the putative functional profile of microbial community exhibited differences among sites, particularly in thermophilic bacteria and metabolic mode, and slightly among treated groups in trophic mode that highlights the adaptive response of microbial community to local environments/stressors. This work confirmed our finding in chapter 2, where corals at the

northern Red Sea is more tolerant than the central region, and provide an evidence that this tolerance was linked to stability of the microbial composition suggesting presence of certain microbial phylotypes fill specific niches that possibly improve thermal tolerance.

4.2. Introduction

The future persistence of coral assemblages is highly depending on corals ability to resist thermal anomalies predicted for the future. Several studies have proven that coral holobiont can locally adapt/acclimate to specific thermal regimes (Palumbi *et al.*, 2014) via various acclimation and adaptation mechanisms (see, DeSalvo *et al.*, 2010; LaJeunesse *et al.*, 2010; Howells *et al.*, 2011; Oliver & Palumbi, 2011a; Barshis *et al.*, 2013; Ainsworth *et al.*, 2015; Dixon *et al.*, 2015; Silverstein *et al.*, 2015). Consequently, corals adapted to extreme and high variable environments (e.g. Arabian Gulf, Red Sea, intertidal reef flat, hydrothermal vents, etc) have received much attention (Hirayama *et al.*, 2007; Oliver & Palumbi, 2011a; D'Angelo *et al.*, 2015; Hume *et al.*, 2015; Schoepf *et al.*, 2015). In particular, corals that thrive in high natural thermal regime have been identified to be high in thermal tolerance, often across regional (e.g. Arabia/Persian Gulf), but also at small spatial scales (e.g. American Samoa) (Oliver & Palumbi, 2011a; Hume *et al.*, 2013) providing opportunities to examine the mechanisms underlying corals resistant to thermal stress.

The variability of corals thermal resistance is driven by several factors including, but not limited to (see chapter1), colony morphology (Marshall & Baird, 2000), symbiont genotypes (Stat *et al.*, 2013), heterotrophic capacity (Grottoli *et al.*, 2006), lipid reserves (Grottoli *et al.*, 2014) and host physiological plasticity (Weis, 2010). Microbial communities associated with corals also play an important functional role in dictating coral fitness (Rosenberg *et al.*, 2007; Bayer *et al.*, 2013a) and acclimation of coral holobiont to

environmental stressors (Reshef *et al.*, 2006; Röthig *et al.*, 2016). It has been reported that coral reefs harbor thousands of microbial phylotypes that differ spatially among sites and across coral species (Sweet *et al.*, 2011b; Lee *et al.*, 2012; Ainsworth *et al.*, 2015), and contribute functionally to coral fitness (Ritchie, 2006), nutrition (Lesser *et al.*, 2007) and nutrients cycling (Raina *et al.*, 2010; Bourne *et al.*, 2016) and defense against pathogens (Brown & Bythell, 2005).

Several studies have documented a dynamic shift of microbial communities associated with corals change responding to biotic and abiotic factors (Lee *et al.*, 2015; Ziegler *et al.*, 2015a; Cardini *et al.*, 2016; Röthig *et al.*, 2016). For example, increase of SST induces change in microbial community composition (Bourne *et al.*, 2008; Littman *et al.*, 2011; Webster *et al.*, 2011, 2016) that may lead to increasing pathogens, and hence the prevalence of corals disease (Remily & Richardson, 2006; Ward *et al.*, 2007; Thurber *et al.*, 2009; Mouchka *et al.*, 2010). Despite this, coral communities inhabiting extreme environments (e.g. hydrothermal vent) have distinct microbial assemblage (Hirayama *et al.*, 2007; Childress *et al.*, 2011) suggesting presence of certain microbial phylotypes (>100) filling a specific environmental niche enabling corals to survive in extreme habitats (Hernandez-Agreda *et al.*, 2016). This highlights the importance of the microbial community that shape the genetic make-up of the coral holobiont, therefore its function, that may improve coral fitness to living in extreme environment.

In addition, both physiological plasticity and genotype identity of *Symbiodinium* spp. are important factors known to influence the thermal tolerance of corals (Reusch, 2014; Ziegler *et al.*, 2015b). For example, corals that harbor clade D symbiont are often more thermal resistant than corals that harbor other symbiont types (Stat & Gates, 2011). Also,

Hoadley *et al* (2015) demonstrated that the photosynthetic efficiency of corals with different symbiont clades varied in their response to changes in SST suggesting that the plasticity of the coral-symbiont physiology is key in determining the degree of tolerance to thermal anomalies (see also Hoadley *et al.*, 2016). Also, the physiological plasticity does not take place only between different coral species, but also within the same coral species harbored the same symbiont type. For example, *Pocillopora verrucosa* increased its photosynthetically light harvesting pigments, thus photochemical efficiency, after cross transplantation experiment among depths (Ziegler *et al.*, 2014; see aslo Gates & Edmunds, 1999). Certainly, symbiont type and its physiological plasticity influence the ecological niche of corals and their capability to populate different environments (Bongaerts *et al.*, 2011; Cooper *et al.*, 2011).

However, the Red Sea experiences highly variable SST and anomalies (see chapter 2), it is populated with extensive health reefs (see; Edwards & Head, 1986) and shows high thermal tolerance toward the north (e.g. Fine *et al.*, 2013). It has been proposed that all present day corals had to recolonize the Red Sea post the Last Glacial Minimum (ca. 6-7kyr BP) and thus, recruiting larvae had to pass the 32 $^{\circ}$C thermal barrier at the southern entrance of the Sea (Braithwaite, 1987; Trommer *et al.*, 2010). Consequently, corals that successfully recruited must be to thermally tolerant to 32 $^{\circ}$C as proposed by Fine *et al.*, (2013). Ubiquitous coral recruitment across the Red Sea has been supported by some population genetic research that demonstrated absences of genetic variation in key species (e.g. *P. verrucosa*) (Robitzch *et al.*, 2015).

Consequently, Fine *et al* (2013) proposed that the northern Red Sea corals (i.e. Gulf of Aqaba) survive below their maximum thermal threshold and this region may serve as a

natural corals refuge. Sawall *et al* (2015) observed variable acclimation potential of *Pocillopora verrucosa* across latitudinal gradients of the Red Sea, and identified the northern Red Sea as an area that exhibited high acclimation potential, while the central and particularly southern Red Sea are less acclimated and exist at their upper thermal margin. The difference in regional tolerance of the Red Sea may refer to acclimation (i.e. physiological) or adaptation (i.e. genotyping) of the coral holobiont rather than the coral host (see Ziegler *et al* 2015b; Hume *et al.*, 2015) however, the role of non-zooxanthellate microbiome remains not well explored.

This chapter experimentally investigates the hypothesis that corals from sites with different thermal histories (i.e. northern and central regions of the Red Sea) have different potential of thermal acclimation/adaptation. Also, it is important to understand the role of the microbiome (i.e. *Symbiodinium* and microbial community) in corals tolerance and whether the non-zooxanthellate microbiome improve corals thermal tolerance or the symbiotic variant is playing the major role in tolerance process. Therefore, the objective of this chapter is to i) investigate physiological status of key coral species inhabiting central and northern Red Sea after being exposed to short-term stress experiment and ii) compare the microbiome composition after thermal stress to see how it changes in a response to thermal stress and between sites experience different thermal regime. The hypothesis is that corals in the northern Red Sea will be less thermally tolerant, but the symbiont genetic variant will be different in both sites. This chapter provide new insight into the holobiont composition associated with coral species that have different thermal tolerances.

4.3. Materials and Methods

4.3.1. Study sites

Based on the analysis of thermal histories across sites (see chapter 2, Table 2-2), two sites with different thermal regimes were selected to investigate corals inherent thermal tolerance using heat stress assay (as previously, Oliver & Palumbi, 2011; Suggett *et al.*, 2012, and see also Fine *et al.*, 2013). Sites were Jeddah, (Thuwal, Al Fahal, 22.2396°N, 38.9634°E) and Hurghada (Abo Galawa, 27.3158°N, 33.8098°E) and experiments were conducted during August and September 2013, respectively. Both sites were offshore patch reefs located away from localized anthropogenic disturbance and with similar geomorphology characteristics and wave exposure (Fig. 4-1).

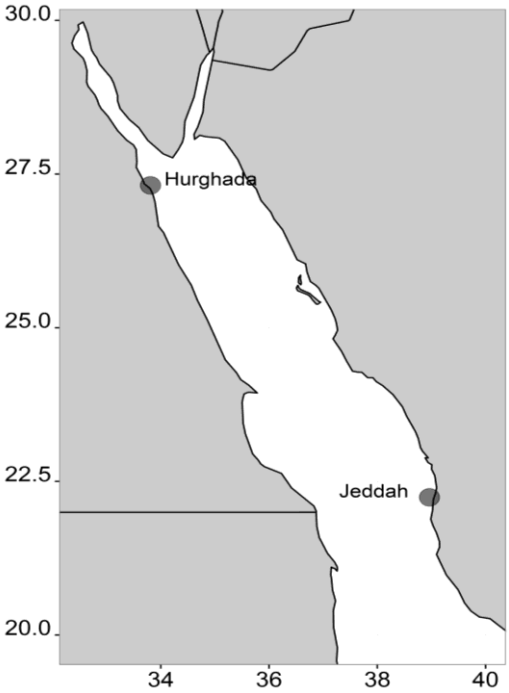

Figure **4-1**. Map of the study sites of two contrasted thermal regimes (Hurghada and Jeddah) where heat stress experiments were carried out

4.3.2. Heat stress assay experiments

At each site, six coral species were chosen and collected from shallow (2-4 m) and deep (15-18 m) water, representing the dominant growth forms; specifically, branching (*Pocillopora damicornis* and *Seriatopora hystrix*), massive (*Porites nodifera* and *Favia favus*), and soft corals (*Xenia umbellate* and *Sarcophyton trocheliophorum*). For each species, replicate samples (n=3) were collected from three randomly selected healthy colonies (>5 m distance apart) placed in pre-labeled zipped bag, then placed in a cool box filled with *in situ* seawater. Upon return to the laboratory, all samples were placed in an

outdoor aquaria shaded and set up to match sampling sites i) light intensity and ii) temperature (ca. 26 °C in Hurghada and 30 °C in Jeddah) for 48hrs (recovery and aquaria acclimation) before assigning to heat stress and control tanks. The acclimation tanks were filled with pre-oxygenated seawater collected directly from the native sampling sites and changed every 12hrs. The temperature (continually logged within aquaria) of the acclimation tanks matched the temperature of the sites from which specimens were collected (ca. 26 °C in Hurghada and 30 °C in Jeddah).

For experimentation, shallow and deep samples were assigned into six shaded outdoor (to match native conditions) 30 gallon aquaria (ca. 136 liter), half of each aquarium was shaded by neutral density filter (Lee Filter, UK) to provide ambient light intensity for deep samples as *in situ*. Each tank was filled with pre-oxygenated seawater collected from sampling sites and stored as *in situ* temperature (ca. 26 °C in Hurghada and 30 °C in Jeddah) in large tank reservoirs, and tanks' water was changed every 12hrs and continually oxygenated by aquaria air pump. Coral samples were equally distributed across experimental tanks, and three tanks each randomly assigned as controls or elevated temperature treatments. Temperature of each tank was controlled using Aqua El-Neo Heaters (Poland) and continually measured by glass thermometer (precision ±0.05 °C) and HOBO loggers in each tank. Based on previous reports of *in situ* bleaching in the Red Sea (see Pilcher & Devantier, 2000), our elevated temperature tanks were 3 °C above Maximum Monthly Mean (MMM) temperature (maximum summer mean as calculated from weekly SST remote sensing data-CoRTAD-v5) for each site (Hurghada 27.8°C, and Jeddah 31.1°C). After 48hrs of aquaria acclimation, temperature was increased to reach Maximum Monthly Mean (MMM; Hurghada 27.8°C, and Jeddah 31.1°C) for treatment aquaria within 24 hours, while

maintained at *in situ* temperature (ca. 26 °C in Hurghada and 30 °C in Jeddah) for control aquaria. Thereafter, the experiment started (time zero) followed by a period of 48 hours to ramp from MMM temperature to elevated treatment (+3 °C above MMM) temperatures (i.e. 1°C/16 hour). After elevated aquaria reached target elevated temperature (i.e. MMM +3 °C), samples were treated for 5-6 days at +3°C where daily mean temperatures for each tank were 26±0.5°C for the controls and 30.8±0.4°C for the treatments at Hurghada, and in Jeddah were 30±0.4°C for control and 34±0.3°C for treatment. Salinity measured twice/day at each tank to ensure constant salinity through the experiment (ca. 40‰ at Hurghada and Jeddah as ambient seawater).

Two metrics were used to quantify photochemical efficiency amongst taxa and sites from the heat stress assays (i) maximum *r*ETR ($rETR^{Max}$, µmol electrons m^{-2} s^{-1}) measured at the start (as quality control measurement for aquarium set up) and end of experimentation, and (ii) Decline of F_v/F_m over time in control versus treatment (as per Oliver & Palumbi, 2011a; Suggett *et al.*, 2012).

Daily measurements of the maximum Photosystem II (PSII) photochemical efficiency (F_v/F_m, as per Oliver & Palumbi, 2011; Suggett *et al.*, 2012) using a Diving PAM fluorometer (Walz GmbH, Germany) was carried out. All settings used were as per Hennige *et al.*, (2008) but modifying the gain where appropriate to ensure the fluorescence signal was in optimum range (F_0, 300–500 instrument units). The Diving PAM fibre-optic probe was attached to a modified holder to standardize the distance (5 mm) between fiber-optic tip and coral surface. Three measurements were recorded from each coral fragment surface at dawn (30 mins after sun rise) and sunset daily. Any signs of coral mortality, e.g. tissue sloughing off the skeleton, was also recorded.

Also, coral photosynthetic responses were assessed using Rapid Light Curves (RLC) at midday of the start and end of the experiment for both control and heat treated samples. A serious of red pulse (n=8) modulated light followed by steps of ascending actinic light every 30 sec (Actinic Intensity= 6, Actinic Light Factor=1) were conducted on each fragment (n=3) for each species (as per Hennige *et al.*, 2008; Suggett *et al.*, 2012). Measurements of the photochemical efficiency at each light step (F_q'/F_m', dimensionless) were multiplied by both the corresponding light intensity (E, μmol photons m^{-2} s^{-1}) and an assumed constant value of 0.5 to account for the proportion of photons to PSII (see Hennige *et al*. 2008) to yield the relative Electron Transfer Rate (rETR, μmol electrons m^{-2} s^{-1}) for each light step. The light-dependency of the ETR was determined for each RLC by fitting values of rETR to E using modified least squares non-linear regression model (see Hennige *et al.*, 2008) as in equation 4-1.

$$\mathrm{rETR} = \mathrm{rETR^{Max}}\ \mathrm{X}\left\{1 - \exp(-\alpha\ \mathrm{X}\frac{\mathrm{E}}{\mathrm{rETR^{Max}}})\right\} \qquad \text{[Eq. 4-1]}$$

Where the terms α describes the light-limited initial slope (dimensionless) and *r*$\mathrm{ETR^{Max}}$ (μmol electrons m^{-2} s^{-1}) describes the maximum relative electron transport rate.

4.3.3. Sample collection

The microbiome of three corals species specifically; *Porites nodifera*, *Pocillopora damicornis* and *Sarcophyton trocheliophorum* (n=3 each) was investigated. Corals specimens were collected and their *Symbiodinium* cladal types and microbial composition were determined. For symbiont identification, coral fragments (<1 cm^2) were collected before assigning corals into experimental tanks and preserved in 2 ml preloaded vials with DMSO-20% buffer. At the end of heat stress experiment, further coral fragments (<1 cm^2) from same coral samples were collected from both control and thermal treated tanks, and

preserved in 2 ml preloaded vial with RNAlater (ThermoFisher, USA) to investigate shifts in the microbial community as a response to heat stress. All collected samples were kept in 4 °C till shipping to University of Essex and then in -20 °C until analysis.

4.3.4. *Symbiodinium* Identification

Symbiodinium clade types associated with the corals species were identified using ribosomal internal transcribed spacer 2 (ITS2) as described in chapter 3. Briefly, corals and associated *Symbiodinium* nucleic acids were extracted using the Wizard DNA prep protocol by Promega (Madison, WI, USA) as per LaJeunesse *et al* (2003). Thereafter, genomic DNA was used to amplify *Symbiodinium* ITS2 region by using ZITS2for and ZITS2clamp primer as designed by LaJeunesse & Trench (2000). The amplified ITS2 regions for each species were aligned against ITS2 amplicon from winter/northern samples (see chapter 3) as a reference symbiont, and then both separated by 30-60% DGGE gel electrophoresis using Bio-Rad, DCode system (California, USA) for 14hrs at 100V (Thornhill *et al.*, 2010). The DGGE gel was stained by sliver nitrate staining solution (Bassam & Caetano-Anollés, 1993) and *Symbiodinium* fingerprints were compared to those that have been identified and sequenced previously in our survey (see chapter 3). The fingerprint of symbiont cladal type did not vary among sites and consequently, no further sequence was carried out for DGGE bands (Figure S11 - Appendix).

4.3.5. 16S rRNA Microbial Community

16S bacterial community sequencing using metagenomics analysis (as described in chapter 3) was carried out on coral fragments following experimentation (samples taken from heat stress and controlled samples). Briefly, extracted genomic rDNA of coral fragment was used for microbial 16S library preparation. Hypervariable V3-V4 regions of bacterial 16S rRNA were amplified using universal 805 reverse and 341F forward primers that attached

with Illumina overhang adaptor (underlined, Illumina, San Diego, CA, USA). The amplicon was cleaned by AMPure XP magnetic bead system (Beckman Coulter, Brea, CA, USA), and then 5 µl of cleaned product was used for indexing PCR for 28 cycle using Nextera XT V2 kit (A&B index kit) (Illumina) according to manufactures manual. Indexed amplicon was further cleaned by AMPure XP magnetic beads and then quantified using FLUOstar Omega microplate reader (BMG Labtech, Germany) using Quant-iT PicoGreen dsDNA assay kit (Invitrogen, USA), and then all samples pooled in equimolar ratios. The quality of the final pooled library was checked on Bioanalyzer (Agilent 2100, Santa Clara, CA, USA), and the library then sequenced on the Illumina HiSeq platform, 2x 300pb paired end by Version 3 chemistry kit at TGAC genomic analysis center (Norwich, UK).

Bioinformatics __ Raw sequences were trimmed using Sickle version 1.33 (Joshi & Fass, 2011) at the default quality threshold (Q20) using paired-end mode. The sequence trimming was carried out at the 3' end, and all sequences either shorter than 250pb or having ambiguous bases (Ns) were discarded. The forward and reserve sequences that passed quality filter were then subjected to error correction using BayesHammer implemented SPAdes v3.7.1, with default settings (Nikolenko *et al.*, 2013; Nurk *et al.*, 2013). The paired-end sequences were aligned and primers removed using the PEAR algorithm implemented in PANDAseq version 1.33 (Masella *et al.*, 2012; Zhang *et al.*, 2014). Paired reads were then de-replicated, sorted by abundance and clustered into OTUs at 97% similarity threshold using Vsearch v1.11.1 (Rognes, https://github.com/torognes/vsearch), and low abundance sequences (<5 occurrence), that more likely representing erroneous sequences, were removed. Taxonomic divisions were assigned as OTU centroids using the RDP classifier (Wang *et al.*, 2007) as implemented in Qiime (Caporaso *et al.*, 2010), with a minimum 0.7

confidence level, and relative abundances of taxa were computed using Qiime's "summarize_taxa.py" script.

4.3.6. Data Analysis

For the heat stress assay, multifactorial ANOVA was performed on $rETR^{Max}$ to investigate the effect of sites, depth and coral species on maximum relative electron transfer ($rETR^{Max}$), while One-way ANOVA was performed on $rETR^{Max}$ (n=3) followed by *Post hoc* Tukey to test the effect elevated temperature on each coral species separately at each site. Further multifactor repeated measures ANOVA was performed on F_v/F_m to test the photochemical yield decline between treated groups and to measure the influence of sites, coral species and depth on F_v/F_m.

To investigate the change of microbial diversity between sites, corals species and treatment groups, choa1 richness estimator, Shannon and inverse Simpson diversity indices were performed on OTUs for all samples. Normality checked was carried out on diversity indices using Shapiro test, and then log or square root transformation was performed if needed. Multifactorial ANOVA was performed on diversity indices to investigate the effect of sites, treatment groups and corals species on microbial diversity. Furthermore, to investigate the separation between microbial community, ordination Principle Coordinate Analysis (PCoA) was plotted using Bray-Curtis dissimilarity resemblance matrix and its dispersions was tested by Permutation Multifactorial Analysis of Variance test (PERMANOVA) (Anderson, 2001). To identify the specificity of microbial species that significantly ($p<0.05$) associated to coral host, sites and treatments, indicator species analysis was performed on OTUs using the indispecies package in "R" (Cáceres & Legendre, 2009). All statistics and plots were conducted on "R" statistical software program (R Developement Core Team, 2015).

Comparative metagenomics is an emerging branch of metagenomics using 16 rRNA data to compare environmental bacterial communities and perform taxonomic-to-phenotypic mapping that was required previously sophisticated knowledge. Herein, the putative functions profile of microbial community were assessed and compared between sites and heat stress groups, using METAGENassist statistical tool for comparative metagenomics web interface for taxonomic to phenotypic mapping (Arndt *et al.*, 2012) (http://www.metagenassist.ca/METAGENassist/faces/Home.jsp). Input file of OTUs (.csv) prepared by summed all distinct OTUs into taxonomical bacteria at genus level (i.e. 6547 OTUs transformed to 954 genus) and then filtered according to interquartile range (Hackstadt & Hess, 2009). Thereafter, the remaining 162 taxa were normalized over samples by sum and range scale as per Röthig *et al* (2016), and microbial community was analyzed by automated taxonomic-to-phenotype mapping tool. The microbial community was assigned to their temperature preference, feeding mode and metabolism using Euclidean distance and average clustering algorithm to visualize the outcome in heatmap. The same analysis using the same settings was performed on microbial community at each site separately to investigate the difference in putative functions between treatment and control.

4.4. Results

4.4.1. Coral heat stress assays

Substantial differences in *r*ETR^{Max} were observed between sites and coral species at each site. Specifically, *P. nodifera* samples showed high thermal tolerance in both sites, but *F. favus* exhibited significant decline (Tukey's, $p<0.001$) in *r*ETR^{Max} of treated samples in Jeddah (Fig. 4-2). *P. damicornis*, similar to *S. hystrix*, were not affected by thermal stress at Hurghada however, heat treatment significantly reduced *r*ETR^{Max} at Jeddah compared to the control samples (Tukey's $p<0.001$, see Fig. 4-2). Similarly, treated *S. trocheliophorum* was tolerant at both sites and was not influenced by heat stress, while *r*ETR^{Max} of treated *X. umbellate* significantly declined at both sites (Fig. 4-2). Data also confirmed that the thermal tolerance of all species was not influenced by depth at both sites, where the change in *r*ETR^{Max} was not significantly ($p>0.05$) changed between depth for each species (Fig. 4-2). Similar to *r*ETR^{Max} results, dark acclimated maximum photochemical quantum yield of photosystem II (F_v/F_m) confirmed that corals at Hurghada were more tolerant than Jeddah for most of the species (Fig. 4-3).

Overall, Hurghada maintained higher photochemical efficiency (i.e. *r*ETR^{Max} and F_v/F_m) in the treatment relative to controls, and hence corals able to thrive above the local threshold, unlike Jeddah which exhibited high decline for most treated corals species. Also, data exhibited differences in photochemical efficiency among coral species where massive corals (i.e. *P. nodifera* and *F. favus*) were more thermally tolerant than branching and soft corals suggesting potential tolerance/susceptibility to different growth forms.

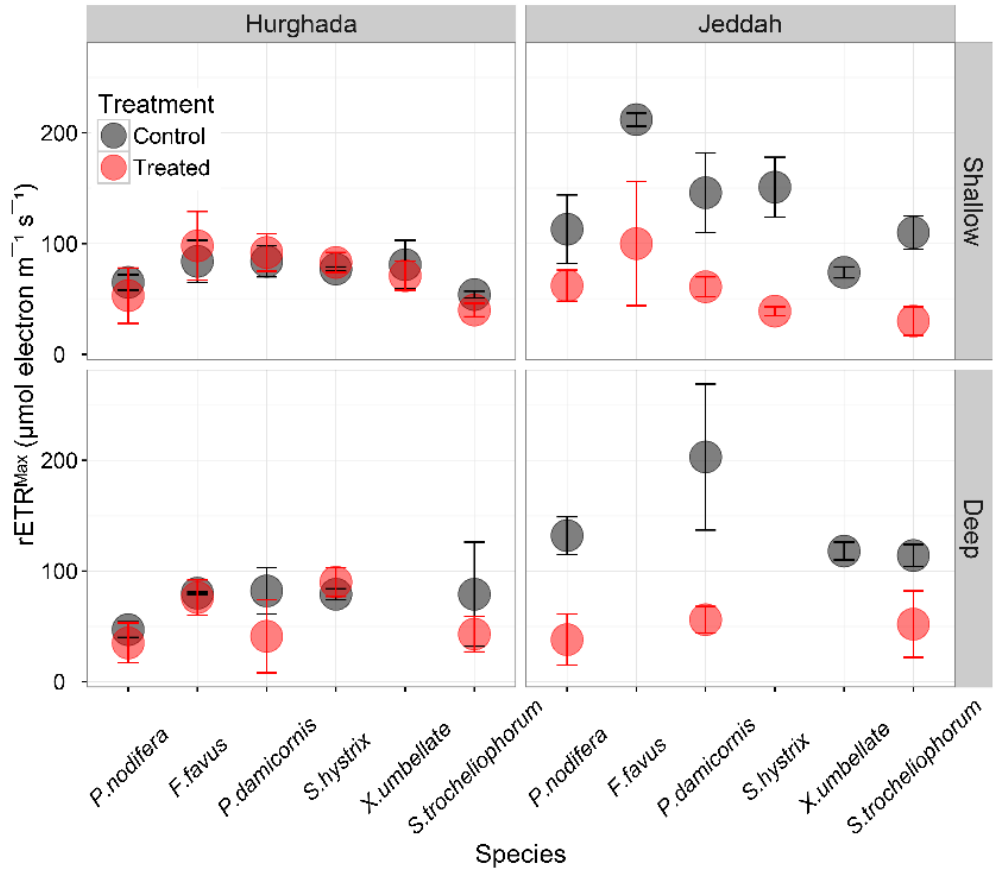

Figure **4-2**. Mean ± SD of maximum relative Electron Transport Rate (*r*ETR^{Max}) of six coral species after thermal stress experiment (+3 °C) in both Jeddah and Hurghada at both depth levels. Plot shows the difference between *r*ETR^{Max} mean in control and treated samples, and data revealed high decline of *r*ETR^{Max} of treated samples in Jeddah in comparison to coral species at Hurghada that showed high thermal tolerance and mean *r*ETR^{Max} remained similar to control ($p>0.05$). *F. favus* and *S. hystrix* in Jeddah as well as *X. umbellate* in Hurghada are missing from deep samples due to difficulty to find them in sampling locality. Also, treated sample of *X. umbellate* in Jeddah is missing due to sudden mortality.

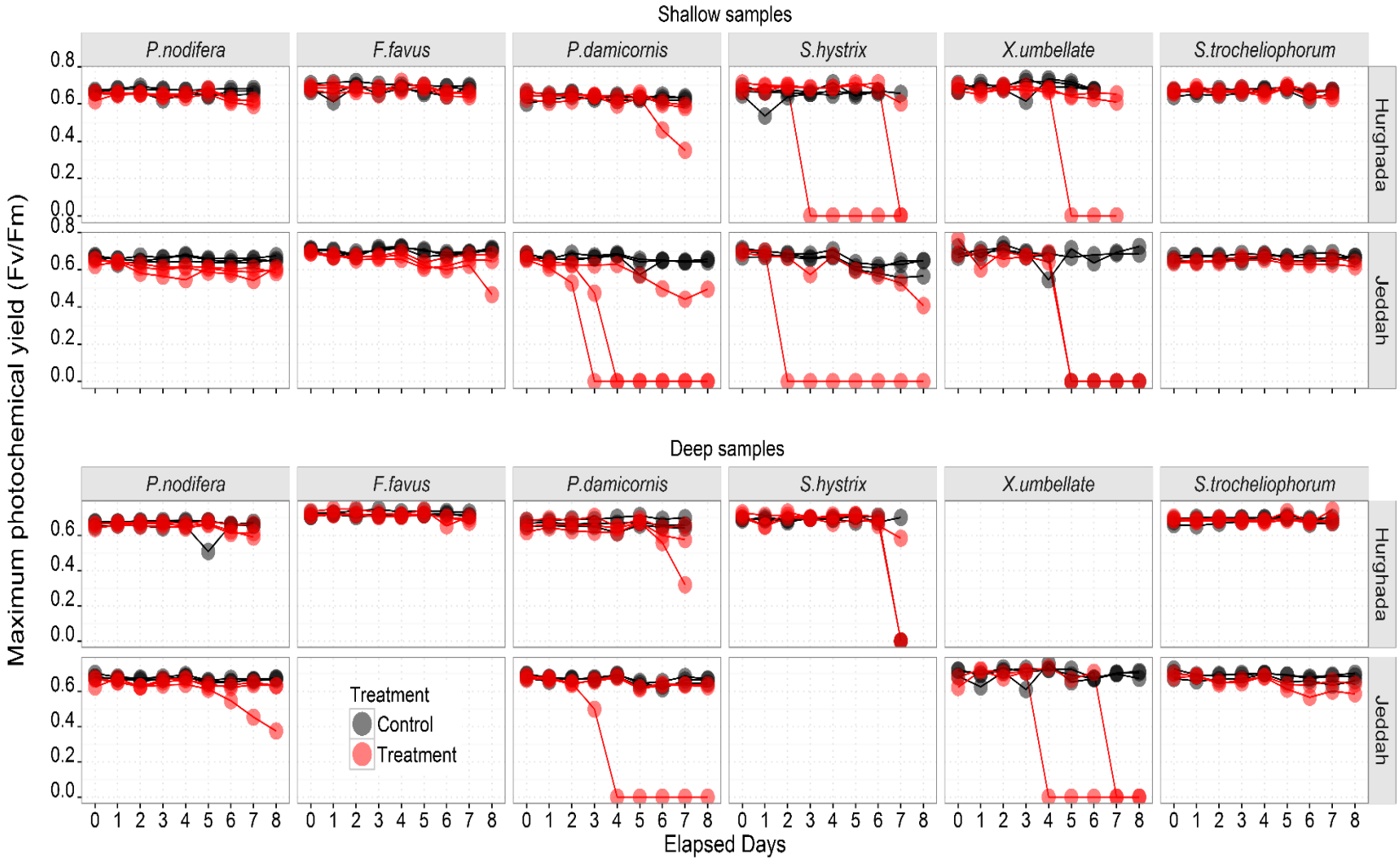

Figure **4-3**. Dark acclimation photochemical yield (F_v/F_m) time series of control and heat stressed fragments (n=3 each) for coral species in both Hurghada and Jeddah at two depth levels. Three replicated of each control and heat stress were plotted presenting the F_v/F_m decline over elapsed days and tested statistically by between subjects two way repeated measure ANOVA using significance value <0.05.

4.4.2. *Symbiodinium* clades:

Genetic characterization of *Symbiodinium* clade ITS2-type associated with coral species identified the same ITS2 types at Hurghada and Jeddah for each corals species, and all were similar to symbiont types recorded in the northern sites during the winter survey (see chapter 3). Specifically, *P. nodifera* harbored C15 clade ITS2 type, while *P. damicornis* and *S. trocheliophorum* harbored A1and C65 variant1 respectively at both Hurghada and Jeddah. This result highlighted that *Symbiodinium* clade types exhibited a high degree of host-symbiont specificity at both sites. Despite this, thermal stress (i.e. treatment) declined photochemical efficiency of corals at Jeddah (e.g. *P. damicornis*), which highlights higher potential acclimation in Hurghada (i.e. northern Red Sea) than Jeddah (i.e. northern Red Sea), therefore lower thermal threshold at Jeddah where corals live in upper thermal maxima.

4.4.3. Microbial community

The sequence of total microbial community of targeted coral species (i.e. *P. nodifera*, *P. damicornis* and *S. trocheliophorum*) at both Hurghada and Jeddah produced 4.25 m reads (ranged from 3150 to 197,199 reads; median= 35633 reads) belonging to 6547 OTUs. However, Proteobacteria was the dominant phylum in all samples, the remaining taxa of microbial community exhibited variations among sites, coral species and treatment groups (Fig.4A). For example, *S. trocheliophorum* was dominated by Spirochaetes at Hurghada (ca. 52% and 30% of control and treated samples respectively), while Firmicutes was dominant at Jeddah (ca. 26% and 10% for control and treated samples respectively). Similarly, the microbial community associated with *P. damicornis* shifted at Jeddah among treatment groups, particularly cyanobacteria (ca. 7% in control and ca. 38% in treated samples), while the microbial community remained similar between treatment groups at Hurghada (Fig. 4-4A).

Taxonomic profile to genus level exhibited that only 13 OTUs composed ca. 44% of total microbial abundance. Endozoicimonaceae was the most abundant taxa (ca.20.5% of total microbial abundance) but changed between treatment groups (Fig. 4-4B). *S. trocheliophorum* associated mainly with *Spirochaeta* spp and Rhodobacteraceace at Hurghada, but *Halomonas* spp. and *Bacillus thermoalkalophilus* were dominant at Jeddah (Fig. 4-4B). The overall taxonomical profile of microbial community varied between sites and coral species, as well as among treatment groups.

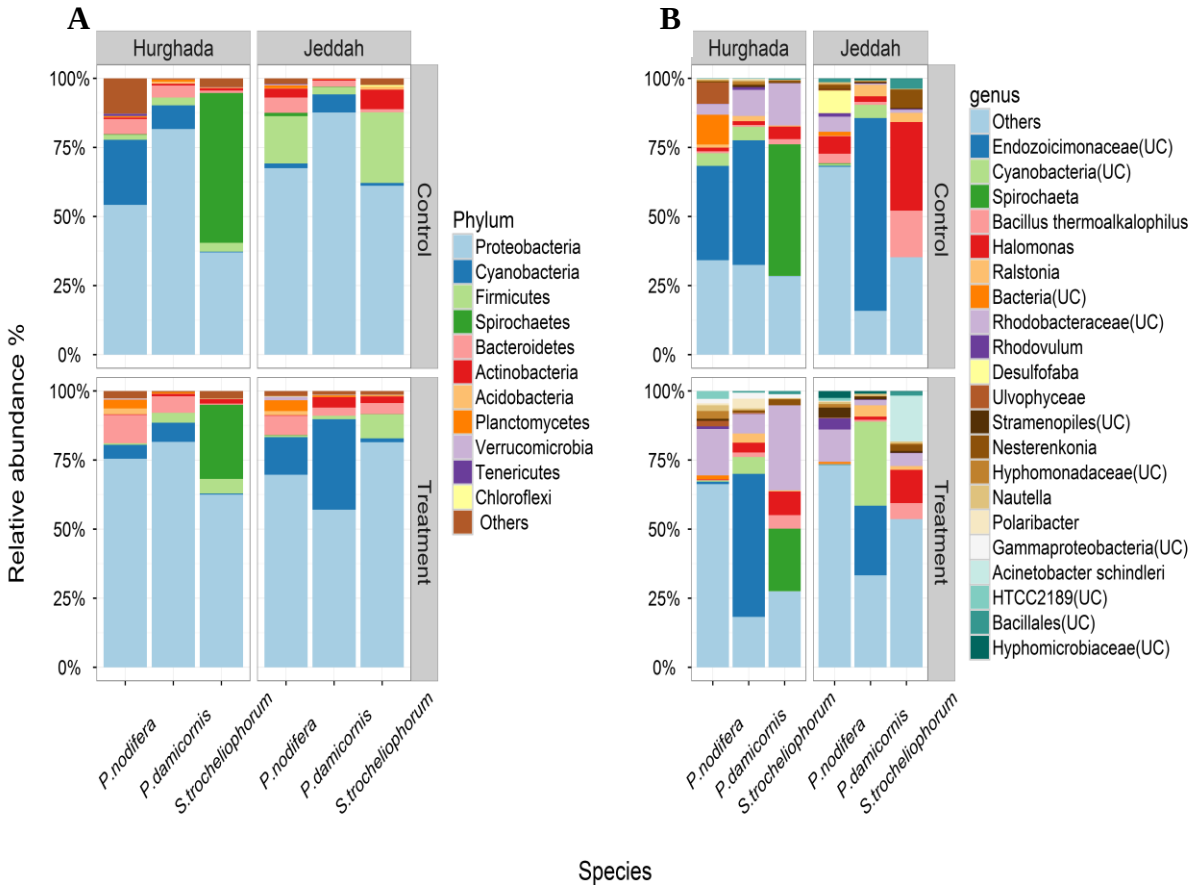

Figure **4-4**. Taxonomic profile of microbial community associated with coral species during heat stress experiment at Hurghada and Jeddah. Taxonomy at phylum level (A) revealed the dominance of Proteobacteria at both sites followed by Spirochaetes and Cyanobacteria, whilst taxonomy to genus level (B) showed high dominance of Endozoicimonaceae at both sites which showed a shift among treatment groups. All unidentified taxa to genus level were denoted UC and lower 1% of relative abundance assigned to "Others" category.

Choa1 richness estimator, Shannon evenness and inverse Simpson diversity indices revealed that the microbial diversity varied significantly among treatment groups and coral species, but did not changed between sites (Table 4-1). Similarly, ANOVA was performed at each site separately confirmed the statistical difference of microbial diversity among coral species within each site (Table 4-1), particularly *S. trocheliophorum* that differed significantly (Tukey's $p<0.001$ for Hurghada and Jeddah) from those associated with *P. nodifera* and *P. damicornis* at both sites. Surprisingly, the heat stress did not influence microbial diversity at Hurghada, while it drove significant changes in microbial diversity at Jeddah (Table 4-1). The overall pattern of microbial diversity did not change among sites, and the influence of coral species on microbial diversity was stronger than the influence of heat stress which driven a significant change in the microbial community at Jeddah, but not in Hurghada (Table 4-1). This highlighted that the decline in photochemical efficiency at Jeddah was associated with change in microbial diversity, unlike Hurghada that remained healthy and its microbial diversity did not change.

Table **4-1**. Summary statistics of analysis of variance (ANOVA) performed on the diversity indices outcome of microbial community associated with coral species that exposed to heat stress at different thermal history sites (Hurghada and Jeddah).

Site	**Variables**		**Choa1 estimator**		**Inverse Simpson**		**Shannon**	
		Df	*F value*	*P value*	*F value*	*P value*	*F value*	*P value*
Between sites	Treatment	1	8.4	p<0.01**	7.7	p<0.05*	12.2	p<0.01**
	Sites	1	1.2	p>0.05	1.7	p>0.05	2	p>0.05
	Corals	2	32	p<0.001***	8.7	p<0.01**	12	p<0.001***
	Treat*Sites	1	4.5	p<0.05*	0.1	p>0.05	0.7	p>0.05
	Treat*Corals	2	1.7	p>0.05	0.2	p>0.05	1.4	p>0.05
	Sites* Corals	2	1.2	p>0.05	2.2	p>0.05	2	p>0.05
	Treat*Sites*Corals	2	2.3	p>0.05	2.1	p>0.05	0.7	p>0.05
Hurghada	Treatment	1	4.7	p>0.05	1.9	p>0.05	3.4	p>0.05
	Coral species	2	15.6	p<0.001***	3	p>0.05	5.9	p<0.05*
	Treat*Corals	2	3.3	p>0.05	0.5	p>0.05	1.8	p>0.05
Jeddah	Treat	1	20	p<0.01**	17.6	p<0.01**	15.9	p<0.01**
	Corals	2	21	p<0.001****	19.4	p<0.001****	10.4	p<0.001***
	Treat*Corals	2	0.4	p>0.05	1.7	p>0.05	0.5	p>0.05

The principle coordinate analysis plot (PCoA) of the microbial community exhibited similar ordination between two sites and microbial community was distinctly separated mainly between coral species and treatment groups, particularly at Jeddah (Fig. 4-5). Statistically, the microbial community structure differed across species, sites and treatment (PERMANOVA, $p<0.001$), but the key factor driving differences was the coral host species (PERMANOVA, $r^2=0.32$), whilst the site and treatment effect were relatively small (Table 4-2). Interestingly, heat stress significantly affected the microbial community at Jeddah more than Hurghada (see Table 4-2). This further confirmed that the relative stability of microbial community structure was associated with higher photochemical efficiency at Hurghada, unlike Jeddah, where decline in photochemical efficiency was associated with change in microbial community structure. Also, the change of microbial community was significantly driven by coral host at both sites which may drive differences in thermal susceptibility among coral species.

Table **4-2**. Summary statistics of PERMANOVA (permutation=999) that performed on microbial community at each site separately. The analysis was performed using Adonis function in vegan package in "R" statistical software using significance level 0.05.

Sites	Variables	Df	Sums of Sqs	Mean Sqs	F.Model	R^2 value	P value
Hurghada	Treatment	1	0.5	0.5	2.8	0.08	0.012*
	Coral species	2	2.8	1.4	8.2	0.47	0.001***
	Treatment*Corals	2	0.7	0.3	2.0	0.12	0.024*
Jeddah	Treatment	1	0.7	0.7	4.2	0.11	0.001***
	Coral species	2	2.7	1.3	8.6	0.44	0.001***
	Treatment*Corals	2	0.9	0.4	2.8	0.14	0.004**

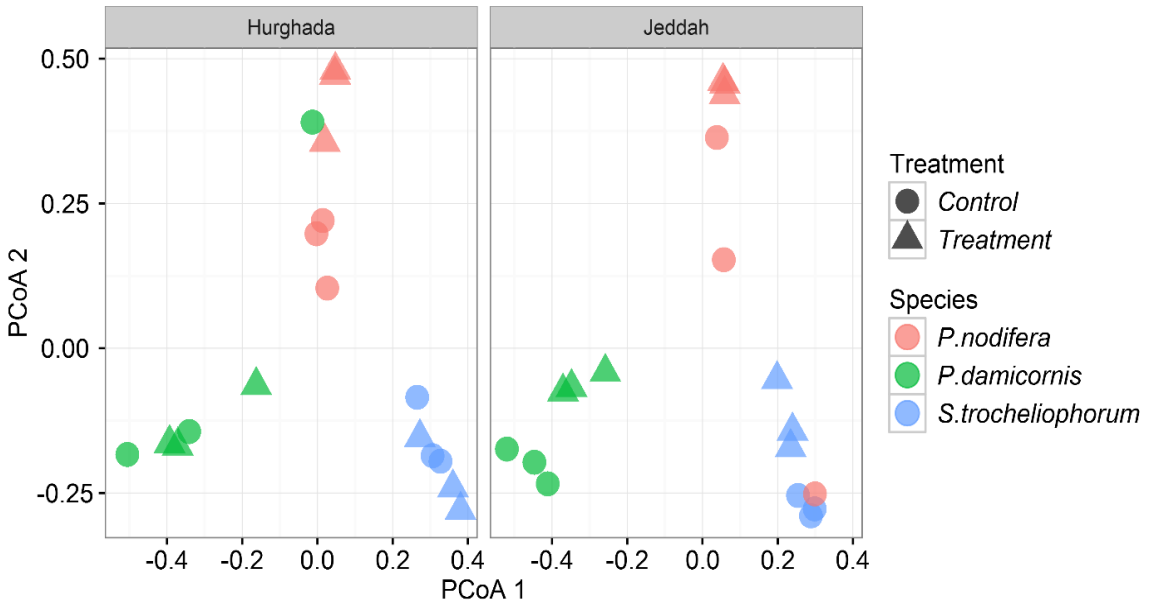

Figure **4-4**. Principle Coordinate Analysis plot (PCoA based on Bray-Curtis dissimilarity) of microbial community associated with three coral species (colors) during heat stress experiment at Hurghada and Jeddah (symbol shape). Plot represents the ordination of microbial community that was similar at both sites and the change of the microbial community was driven mainly by coral host. Also, the heat stress influenced the microbial community in treated samples was higher in Jeddah (i.e. treated groups clustered separately from the control) than Hurghada.

*Associated species*_ To investigate the specificity of microbial OTUs to the studied factors (i.e. sites, corals species, treatment groups), indicator species analysis was performed on OTUs. The microbial specificity to corals species was much higher than those specific to sites and treatments groups. Site had a low impact on microbial community (4% at Hurghada and 3% at Jeddah of total OTUs) and composed ca.18.6% at Hurghada and 13% at Jeddah of total microbial community abundance. The key microbes differed at each site where Hurghada was dominated by Rhodobacteraceae (ca. 10.5%), Cyanobacteria (Ulvophyceae-ca. 8.5%), Polaribacter (ca. 8%) and γ-proteobacteria (ca. 7.1%), while Jeddah was dominated by Delta-proteobacteria (Desulfofaba ca. 12.5), Hyphomicrobiaceae (ca. 7%), Cyanobacteria (Stramenopiles ca. 6%).

Similarly, the microbial community specific to treatment groups differed between control and treated samples. Specific microbes to treated samples composed ca.18% and 19%

of total microbial abundance at Hurghada and Jeddah respectively, whilst those microbes specific to control group composed <1% of total microbial abundance in both sites (Fig. 4-6A). Treated samples were dominated by cyanobacteria (Stramenopiles, ca.11%), Rhodobacteraceae (ca. 9.5%) and Hyphomicrobiaceae (ca. 7.6%) at Jeddah, whilst Hurghada was dominated by Rhodobacteraceae (ca. 30%), unclassified γ-proteobacteria (ca. 10%), and interestingly associated with *Bacillus thermoalkalophilus* (ca. 9% particularly in *S. trocheliophorum*) (Fig. 4-6B). This data highlighted the response of microbial community to increase of water temperature, noticeably Hurghada that was specific to γ-proteobacteria and *Bacillus thermoalkalophilus.* This later bacterial taxon (i.e. *Bacillus thermoalkalophilus*) is well known as extremely thermally tolerant bacteria (Tang *et al.*, 2006).

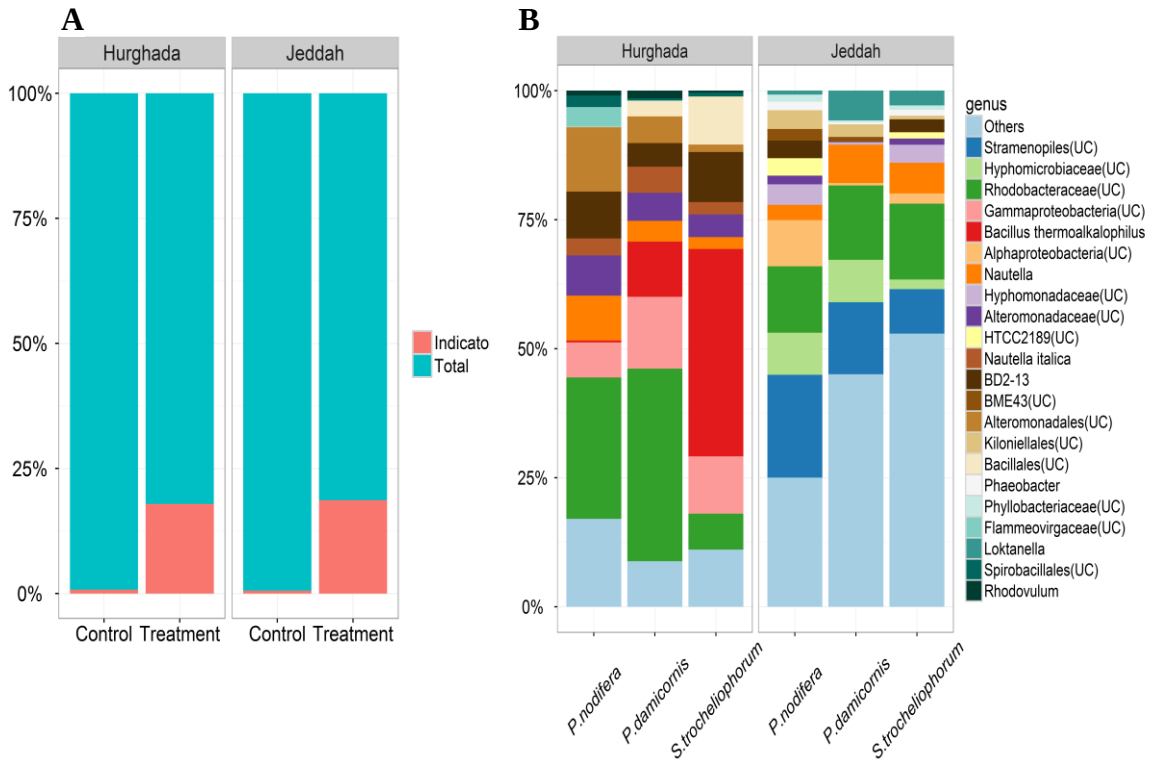

Figure **4-5**. The relative abundance of microbial community significantly specific to treatment groups samples at both sites. Bar plot (A) represents the contribution of indicator species to total microbial community where treated corals was specific to 18% and 19% of total community abundance, while control composed <1% of total abundance. Accordingly, the community structure and its relative abundance of treated samples only were plotted at genus level (B). Taxa below 1% of relative abundance were assigned to "Others" category and unclassified taxa to genus level were denoted UC.

Conversely, corals-specific microbial species comprised a high percentage of total microbial abundance and was highly different between coral species. Specific microbes to *P. nodifera* composed ca.51 and 43% of total abundance at Hurghada and Jeddah respectively (Fig. 4-7A) consisting mainly of Endozoicimonaceae (ca.27%), unclassified bacteria (10%) and Cyanobacteria (ca. 8.5%) at Hurghada. No single taxa dominated the microbial community at Jeddah (e.g. Deltaproteobacteria composed ca. 6%). The indicator species associated with *P. damicornis* composed ca. 68% at Hurghada and 72% at Jeddah (72% and 75% were Endozoicimonaceae respectively), while the abundance of specific microbial community to *S. trocheliophorum* were 67% at Hurghada (Spirochaeta - ca. 51%; Endozoicimonaceae - ca. 33%), and ca. 23% at Jeddah that dominated by *Halomonas* spp. (ca. 37%) and *Bacillus thermoalkalophilus* (ca.22%) (Fig. 4-7B).

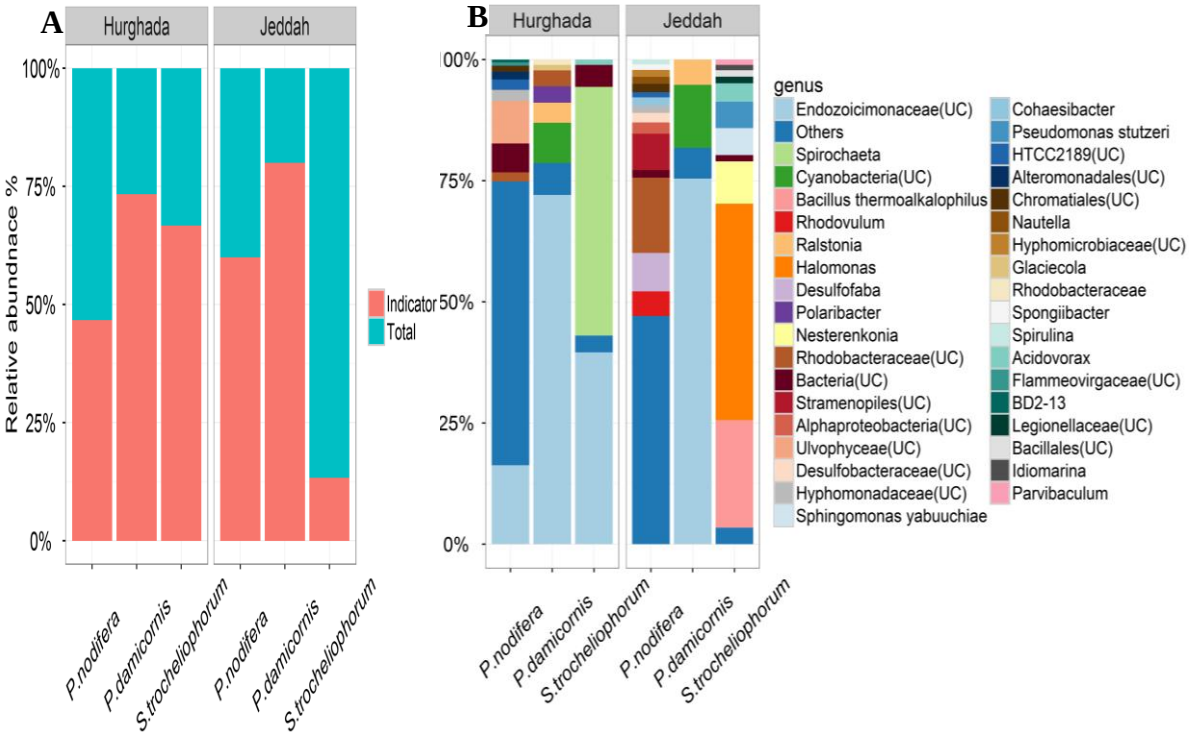

Figure **4-6**. Taxonomy and relative abundance of indicator species analysis that significantly specific to coral taxa ($p<0.05$) at both Hurghada and Jeddah. The contribution of indicator species in total relative abundance (A) of microbial community varied among species. Also, barplot (B) represent the composition of indicator species which exhibited difference in species-specific among coral taxa.

The overall indicator species analysis showed higher specificity of microbial community to coral species suggesting different core microbiome contributing to each coral holobiont. In particular, family Endozoicimonaceae that were associated with healthy corals at Hurghada, and Rhodobacteraceae and cyanobacteria that were associated with treated samples at both sites.

Microbial Functional profile __To assess putative function of microbes associated with corals, the microbial communities were classified according to temperature preference, trophic mode and metabolism. The relative abundance of thermophilic bacteria increased from ca. 10.6% to 16.1% in corals from Jeddah that were treated with elevated temperatures and mesophilic bacteria increased from ca. 11.8% to 15.5% (Fig. 4-8). Conversely, the relative abundance of thermophilic bacteria was much lower at Hurghada comprised 0.1% at both control and treated samples, while mesophilic bacteria increased from 8% in control to 12% in treated samples (Fig. 4-8).

The predominant trophic mode of the microbial community was mainly unknown (ca. 80%) and the remaining did not change between treatment groups at both sites however, slight upregulation in heterotroph and phototroph bacteria in treated samples was observed (Figure S12 – Appendix). Meanwhile, the putative metabolic functional profile of microbial communities differed among sites. At Hurghada, there were relative high abundance of bacteria that ammonia oxidation, stores polyhydroxybutyrat bacteria, nitrite and sulfate reducer, nitrogen fixation and dehalogenation bacteria (Fig. 4-9). Conversely, microbial community associated with corals in Jeddah upregulated streptomycin producer, sulfide oxidizer, atrazine metabolism, naphthalene degrading, sulfur metabolizing, chitin

degradation, xylan degrader, chlorophenol degrading and degrades aromatic hydrocarbon bacteria (Fig. 4-9).

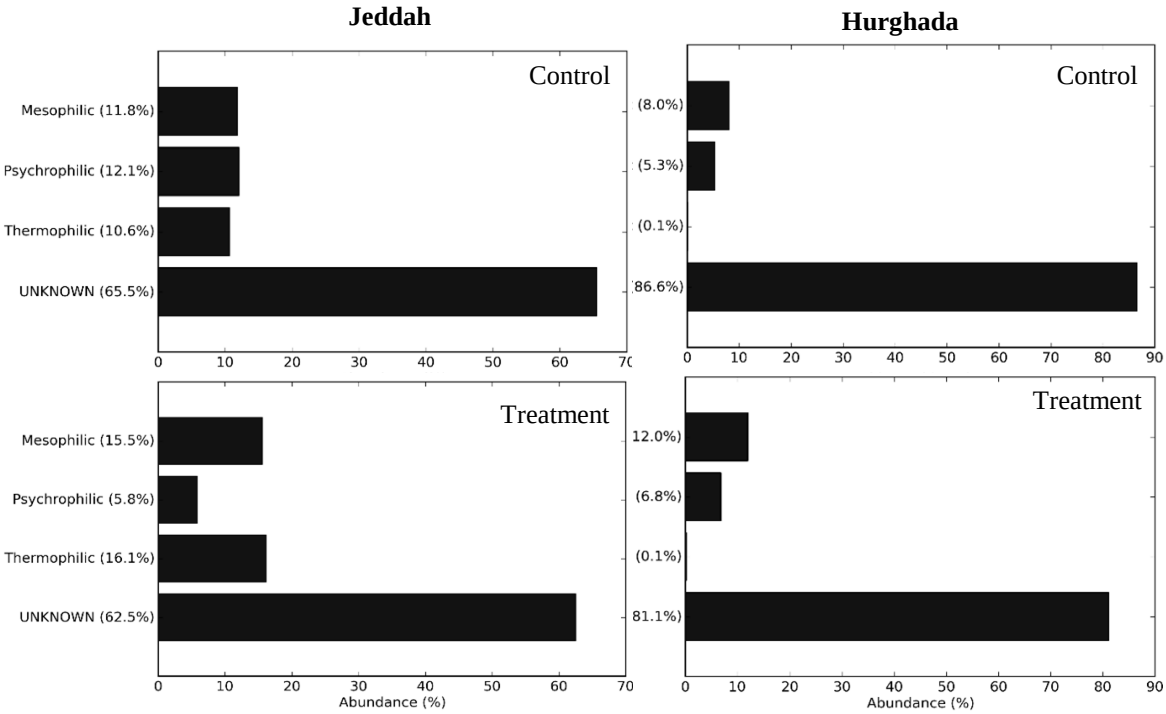

Figure **4-7**. The putative temperature preference of microbial community associated with treatment groups of corals within the experimental sites (i.e. Hurghada and Jeddah). Data demonstrate the relative percentage of bacteria according to its temperature preference based on taxonomic-to-phenotypic tool in METAgeneassist comparative metagenomics web interface.

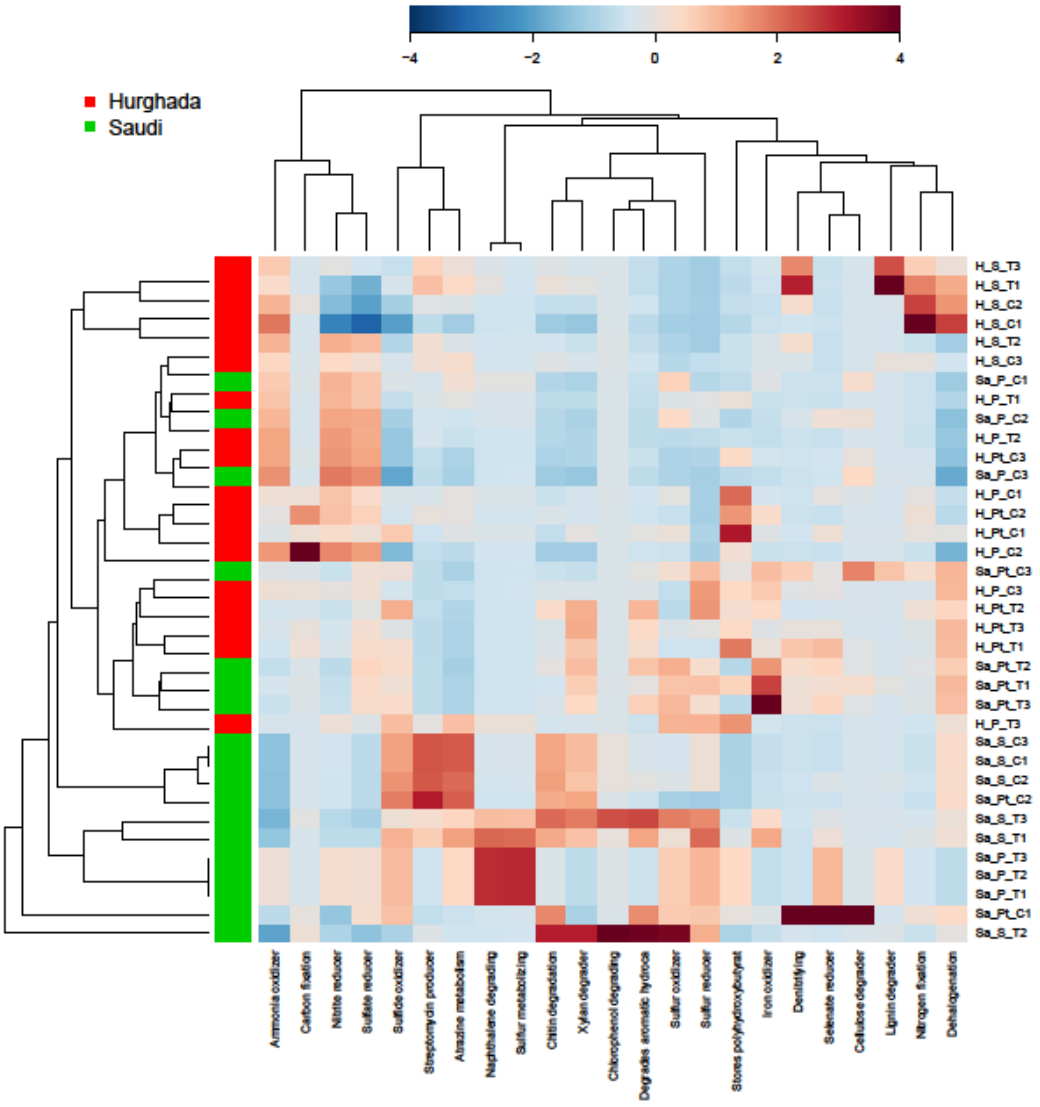

Figure **4-8**. Heatmap represents the putative metabolic functional profile of microbial community associated with corals species (*P. nodifera*=Pt, *P. damicornis* =P and *S. trocheliophorum*=S) for control (C) and treated (T) samples at both sites (green and red index). Data produced by taxonomic-to-phenotypic function in METAGENassit web interface and calculated based on Euclidean distance matrix and average clustering algorithm. Heatmap is displayed by relative abundance of summed OTUs at genus level where it ranged from blue to red color scale in log scale bar.

4.5. Discussion

Reef-building corals living in extreme and variable environments are considered "natural laboratories" to investigate the acclimation and adaptation mechanisms that improve corals to survive in temperature predicted for the future. However, the Red Sea is one of extreme environments, it remains not well explored and little research has been carried out (see, Berumen *et al.*, 2013). Herein, the tolerance of key coral species inhabiting different thermal regimes was experimentally examined to determine the response of the microbiome composition to short term thermal stress.

Our results confirm the findings raised in chapter 2, based on analysis of natural observations of photochemical efficiency to heat anomaly patterns, that there is spatial difference in thermal stress sensitivity from north to south. Corals at the northern Red Sea (i.e. Hurghada) maintained thermal tolerance in comparison to the central Red Sea (i.e. Jeddah) which exhibited a decline in photochemical efficiency (i.e. $rETR^{Max}$ and F_v/F_m) after being exposed to heat stress (3°C above local summer mean) however, they house the same symbiont clade type. This result support the assumption of Fine *et al* (2013) that corals in the Red Sea have the same thermal threshold (>32°C). The water temperature was elevated to 31.1 °C at Hurghada (+3°C above SST summer mean, see methods section) and corals remained thermally tolerant, while Jeddah heated to 34°C but corals were thermally susceptible. This highlights that the northern Red Sea exist below its thermal thresholds and then, have more way to move until corals reach their thermal maxima, unlike Jeddah that thrive near its upper thermal threshold limits and increased water temperature can cause coral bleaching. This highlights that corals in the northern Red Sea are less susceptible to global warming than central Red Sea, and then may serve a potential coral refugia (see chapter 5).

However, the thermal tolerance of reef-building corals is often determined by the symbiont genetic variant (i.e. clade type) (e.g. LaJeunesse *et al.*, 2014), this does not appear to be the adaption/acclimation strategy of investigated corals in the Red Sea. Our results revealed that coral hosts harbored the same symbiont clade types at both experimental sites (*P. nodifera* - C15 clade, while *P. damicornis*- A1 clade, and *S. trocheliophorum*- clade C65 var1) despite their relatively differences in thermal histories (see also chapter 3).

This highlights two important issues; First, the importance of symbionts phenotypic plasticity, not only genetic variants, which enable corals to survive in different thermal/environmental niches. Ziegler *et al* (2014) observed increase in photosynthetic pigments and cell density of symbionts hosted by *Pocillopora verrucosa* in the Red Sea after changing the light intensity. Also, Sawall *et al* (2014) found that *P. verrucosa* harbored prominently A1 ITS2 type across the Red Sea latitudinal gradients however, it expressed different metabolic rates, therefore acclimation, across latitudes (Sawall *et al.*, 2015; sea also Parkinson & Baums, 2014).

Second, the role of host appears to drive the cladal response rather than the environmental conditions. Bhagooli *et al* (2010) reported difference in thermal tolerance of *in hospite* versus cultured *Symbiodinium* suggesting the coral host may provide symbiont with a degree of protection from environmental stressors. Bellantuono *et al* (2012) reported stability of *Symbiodinium* clade and bacterial community during short-term heat stress experiment on *Acropora millepora* in the Great Barrier Reef suggesting that the host physiological plasticity is the key mechanism of acclimation as previously reviewed in Weis (2010). Howells *et al* (2016) found that the persistence of coral population in long term of thermal exposure as in Arabian/Persian Gulf likely determines the evolutionary rate of both

symbiotic partners therefore, their thermal tolerance. Overall, the phenotypic plasticity of both coral host and its symbiont cannot be neglected in thermal tolerance/susceptibility model to obtain accurate predictions (van Woesik *et al.*, 2010).

The heat stress experiments also revealed differences in the susceptibility of coral species and growth forms contrasting the field observations pattern recorded in chapter 2 (see Figure 2-9). The experimental data provided here supported the common hypothesis that massive corals (i.e. *P. nodifera* and *F. favus*) are more tolerant of thermal stress than branching species. Field observations of the Western Indian Ocean have shown branching taxa (such as *Acropora* and *Pocillopora)* to be more susceptible to thermal stress than massive taxa (West & Salm, 2003; McClanahan *et al.*, 2007a). The morphological variations in bleaching susceptibility may contribute to differences in thermal tolerances (Baird & Marshall, 2002) via tissue thickness that play photo-protective role (Dimond *et al.*, 2012), and/ or marked differences in colony size and age (Loya *et al.*, 2001). These differences in thermal tolerance may also be attributed to mass-transfer rate (i.e. potential exchange rate of gases and metabolites across corals' layers) as suggested by van Woesik *et al* (2012). Overall, the thermal susceptibility/tolerance of corals varied among coral taxa as well as sites. It has been proposed that the microbiome beyond that of the symbionts may play a functional role in defining coral holobionts' susceptibility to environmental stress (Rosenberg *et al.*, 2007).

Within our study, the results indicated that the coral host is the major driver of the associated microbial community. This finding supports those of Chu & Vollmer (2016) who concluded similarly across sites within the Caribbean. Host specificity is not confined to reef corals, van de Water *et al* (2016) determined that Spirochaetes dominated the microbial community associated with the red coral (*Corallium rubrum*) across broad geographical scale

in the Mediterranean Sea. La Rivière *et al* (2015) found bacterial-host specificity irrespective to seasonal change for gorgonians within the Mediterranean Sea. It therefore appears that each host may well provide a different niche for associated microbes and then, there are core microbial communities that are unique to each coral host potentially playing specific roles in determining the fitness of a holobiont (Ainsworth *et al.*, 2015; Hernandez-Agreda *et al.*, 2016), and therefore different susceptibility to environmental stressors.

The microbial community composition of the current study varied among coral species and treatments groups. Interestingly, the thermal tolerance of Hurghada coincided with stability of microbial diversity among treatment groups, unlike Jeddah where heat stress influenced microbial diversity significantly. Furthermore, the decline in photochemical efficiency in thermally stressed *P. damicornis* at Jeddah associated with microbial composition shift, (particularly dominance of cyanobacteria and reduced of Endozoicimonaceae), while the microbial composition remained similar between treatment groups at Hurghada. This highlights the importance of microbial consortia and its role in coral fitness as proposed by Rosenberg *et al* (2007). Gilbert *et al* (2012) found severe tissue loss and decline in photochemical efficiency in *P. damicornis* which treated with heat stress and antibiotics combined, while heat treated samples without antibiotic exhibited only photochemical efficiency decline. Therefore, disruption of the microbial community changes the resistance capability of the holobiont where changes may be a positive beneficial response (reviewed in Krediet *et al.*, 2013), but also much evidence suggests that a change can bring about lethality through the proliferation of pathogens (Sussman *et al.*, 2006; Bourne *et al.*, 2008; Webster *et al.*, 2011; Kramarsky-Winter *et al.*, 2014). Lee *et al* (2016) also observed a microbial shift associated in *Acropora muricata* from predominance of γ-Proteobacteria to

pathogenic taxa under heat stress, and the shift was correlated to changes of chemical composition of the mucus suggesting it is the key factor in microbial shift.

Consistently, both taxonomical profile and indicator species analysis indicated that Endozoicimonaceae (γ-Proteobacteria) was specific to all corals species. This bacterial taxa is associated with healthy corals (Kvennefors *et al.*, 2010), and has been assumed to have an important role in coral health as it exists in aggregation within gastroderm close to the symbiont (Bayer *et al.*, 2013a). Neave *et al* (2016) confirmed that and found Endozoicimonaceae associated with *Stylophora pistillata* and *Pocillopora verrucosa* across different global bioregions, but each coral species harbored different phylotypes referring that to host reproductive strategy (i.e. vertical versus horizontal transmission). However, the functional role of Endozoicimonaceae remains unknown, it can be identified as core taxa of microbiome associated with corals (reviewed in Bourne *et al.*, 2016).

The putative functional profile of the microbial community associated with corals indicated the presence of thermophilic and mesophilic bacteria that represented a higher percentage within Jeddah as compared to Hurghada. This may reflect acclimation of corals to survive in high ambient temperatures in Jeddah (31°C) as reviewed in Canganella & Wiegel (2014). Despite this, Jeddah was more thermal susceptible than Hurghada suggesting that the thermophilic bacteria is not necessarily determining corals thermal tolerance, or their functional performance were deactivated. Our results support the later assumption where upregulation of streptomycin (i.e. antibiotic) producing bacteria was observed in Jeddah, which inhibit may thermophilic bacteria activities. This was tested by Tosi *et al* (2007) who reported susceptibility of *Streptococcus thermophilus* to several antibiotics.

An interesting outcome from this study is the trophic mode shift of the microbial community among treatment groups at both sites, where thermally treated coral samples exhibited upregulated heterotrophic and phototroph bacterial community. The relative increase of heterotroph bacteria is a sign of corals mucus production as a response to heat stress (Wooldridge, 2009), where the polysaccharide nature of the mucus act as organic carbon source for heterotrophic bacteria, and therefore opportunistic bacteria colonization takes place (Dinsdale & Rohwer, 2011). Therefore, increase of mucus production here is considered a defensive strategy against environmental stressors (Brown & Bythell, 2005), that coincide with microbial shift resulting from change of mucus chemical composition as reported by Lee *et al* (2016).

Meanwhile, the upregulation of phototrophic bacteria that increased as a response to heat stress confirm our findings in chapter 3 where *Erythrobacter* was more dominant at the warmest site (see Figure S10 - Appendix). The phototroph bacteria appear to have a supplementary nutritional role to coral host to compensate the reduction of photosynthesis assimilation via symbionts during heat stress. However this assumption needs to be further investigated and proven on bacteria involved in this study, similar microbiota associated with corals leak organic/photosynthetic that support the energy needs of the hosts and appears to be regulated by the host as *Symbiodinium* and cyanobacteria (Muscatine *et al.*, 1981; Trench, 1993; Lesser *et al.*, 2004, 2007; Venn *et al.*, 2008; Yellowlees *et al.*, 2008). Furthermore, Morrow *et al* (2014) reported increase of photosynthetic bacteria associated with corals inhabiting hydrothermal vents in Papua New Guinea, suggesting nutritional benefits to corals enhancing their growth rate under extreme warm and acidic water. This confirms the findings in surveyed sites in chapter 3 suggesting a new hypothesis to be tested.

The overall metabolic profile of bacteria exhibited difference among sites, but it is hard to interpret the pattern due to its complexity and cross linking roles. Hurghada was dominated by carbon/nitrogen fixation and ammonia/sulfide oxidizers indicating no stress signs as these are common metabolic pathway for bacteria (Raina *et al.*, 2009; Sharp & Ritchie, 2012; Ceh *et al.*, 2013) however, carbon/nitrogen fixation may be beneficial to corals and providing food source for the host (Lesser *et al.*, 2007; Yellowlees *et al.*, 2008). Conversely and despite no obvious impact of anthropogenic stressors at Jeddah, upregulation of aromatic and hydrocarbon compounds degrader bacteria suggests oil pollution. This may be a result of an extensive discharge of untreated and illegal wastewater into adjacent reef area at Jeddah city (Al-Farawati, 2010; reviewed in Ziegler *et al.*, 2015a) and water currents carry it northward to Thuwal (i.e. our sampling location – 100 km north Jeddah city). Also, upregulation of xylan degrader (i.e. consume celluloses cell wall of plants and algae - see Chassard *et al.*, 2007) and Streptomycin producer (i.e. antimicrobial production defending invasion of pathogen properly- see; Shnit-Orland & Kushmaro, 2009) at Jeddah highlights that corals were under stress.

To conclude, corals in the northern Red Sea appeared more thermal tolerance than the central Red Sea confirming our remote sensing findings in chapter 2 suggesting regional potential to serve as corals refugia. The difference in regional coral tolerance was not determined by symbiont genotyping but was linked to stability of microbial composition at Hurghada under heat stress, unlike Jeddah. This highlights presences of core microbiome filling specific thermal niche that have not been influenced by heat stress and improved coral tolerance at the northern Red Sea. Also, the putative functional roles provide a new

hypothesis to be examined on possible functional roles of these microbial communities bring to survival to transit heat stress events.

Chapter 5

5. *General Discussion*

Reef-building corals have declined worldwide being influenced by frequent bleaching episodes induced by thermal anomalies (Hoegh-Guldberg *et al.*, 2007; De'ath *et al.*, 2012). Fortunately, corals that thrive naturally in high temperature environments have been identified as having a potential to be more resistant to thermal stress (Oliver & Palumbi, 2011a; Howells *et al.*, 2016) and therefore, have taken the focus of much recent research (e.g. Schoepf *et al.*, 2015). Those corals inhabiting extreme environments that are predicted to be more common place in the future not only serve as a genetic reservoir, but may also provide insight in to how corals may adapt to mitigate the predicted scenarios of global warming. Consequently, identifying the locations where corals naturally experience high temperatures is a priority for global conservation and management.

The Red Sea is an extreme environment (Moustafa *et al.*, 2013) making it a model system to investigate the acclimation and adaptation mechanisms, but as compared to other systems in the Caribbean or on the Great Barrier Reef, there has been relatively little published research within the region (Berumen *et al.*, 2013). Research presented here fills key gaps in existing knowledge across different biological disciplines by examining environmental variability (i.e. SST, Chl-*a* and K_d) during the last three decades, the characteristics of past bleaching episodes, and the composition of the functional components of key coral species (i.e. coral physiology and genetic variation between symbiotic zooxanthellae and of the associated microbial community). These approaches are combined to describe the tolerance/susceptibility of reef-building corals to thermals anomalies in the Red Sea.

Remote sensing data showed variability of Sea Surface Temperature (SST) across latitudinal gradients which declined at higher latitudes (i.e. the northern Red Sea). Conversely, thermal anomalies (i.e. DHW) were higher in the northern Red Sea compared to the central/southern Red Sea, but corals did not bleach in this region despite the higher degree heating weeks (ca.15 °C-weeks) (see chapter 2, Figure 2-5). This pattern contradicts the global pattern where massive bleaching is most often observed after 4 °C-weeks and widespread mortality generally occurs following 8 °C-weeks (Liu *et al.*, 2006; Eakin *et al.*, 2010). During experimentation, corals from the northern Red Sea maintained higher photochemical efficiency and appeared more thermally tolerant than the same species (housing the same clade types) from the central Red Sea following exposure to +3 °C above local SST summer means (see chapter 4). Therefore, remote sensing and experimental data confirmed that the northern Red Sea is extremely thermally tolerant when compare to the central-southern Red Sea and thus findings presented here are consistent to recent published research (see; Fine *et al.*, 2013; Sawall *et al.*, 2014, 2015).

Fine *et al* (2013) concluded that corals in parts of the Red Sea have a similar thermal threshold (>32°C) and that the Gulf of Aqaba is a thermal refuge for corals. Our data extend this finding to include areas beyond the Gulf of Aqaba into the entire northern Red Sea region but particularly the Egyptian coast where summer SST mean ranging between 28-29°C (see, Table 2-2). It means that the northern Red Sea corals exists below their thermal threshold (>32 °C) and may act as a refuge if SST increases by 2-3°C at 2100 as proposed by IPCC (2014). Our conclusions were supported by data collected in 2015, the warmest year in earth's recorded history, but where corals within both the Gulf of Aqaba and the northern Red Sea remained bleaching free despite high DHW (>8 °C-weeks), whereas bleaching was

apparent at Jeddah and further increased southwards (unpublished data) suggesting that corals of the central and southern Red Sea exists much closer to their thermal maxima. Other environmental factors may also play a role and Cacciapaglia & van Woesik (2016) identified the northern Red Sea as a more turbid refugia system based on a hierarchical Bayesian model where the interaction between turbidity and tides mitigate the effect of increasing SST often combined with high light intensities.

This finding emphasis the importance of implementing strategic conservation plans for the northern Red Sea as a critical region that serves as a possible refugia. Most of the Egyptian Red Sea coast is protected (MPAs) but the management and conservation plans of the Red Sea Protectorates lack a strategy to deal with global warming and mass bleaching. The whole northern Red Sea region, as possible refugia, needs a regional policy to minimize non-climatic stressors (i.e. so to enhance water quality, improve fisheries management policies, maximize the conservation value of ecotourism, and enhance environmental education and awareness) to maximize its conservation significance. Initiation of global network of coral refugia is critical for reefs conservation, where long distance translocation from refuge environments could represent one future for coral reef conservation (Van Oppen *et al.*, 2015). But others have demonstrated that corals are often adapted to local environmental conditions and therefore despite being thermally tolerant, competitiveness and tolerance might be lost if corals are moved from their local setting (D'Angelo *et al.*, 2015).

The thermal resistance of reef-building corals may be linked to 1) various environmental variables, 2) physiological plasticity of either host or *Symbiodinium* or both, 3) genotype of hosted symbiotic variants and associated microbial community as summarized in conceptual model in chapter 1 (see; Figure 1-5). Initially, the hypothesis was that the coral

resistance to thermal stress in the northern Red Sea is related to specific genetic variants of dominant *Symbiodinium* spp. as previously reported (Baker, 2002; Stat & Gates, 2011; Silverstein *et al.*, 2015; Boulotte *et al.*, 2016). The *Symbiodinium* clades dominant in corals sampled within this research are cosmopolitan (see; Ulstrup *et al.*, 2006; Sampayo *et al.*, 2007; Fitt *et al.*, 2009; Hume *et al.*, 2013; Stat *et al.*, 2013), and do not appear to be specific to warmer seas and do not appear to play a role in thermal tolerance. However, there are several possible reasons to be cautions when inferring tolerance of a coral holobiont and two main issues should be considered.

Firstly, the resolution of genetic marker that has been used for *Symbiodinium* identification. Barbrook *et al* (2006) reported that non coding region of minicircle chloroplast ($psbA^{ncr}$) differed within the same symbiont clade types, but geographically distant, suggesting that $psbA^{ncr}$ might be a good genetic marker to resolve closely related symbiont types. Similarly, LaJeunesse & Thornhill (2011) found that genus *Montipora* in Hawaii was dominated by only one symbiont type after using $psbA^{ncr}$ however, ITS2 profile was phylogenetically different suggesting that $psbA^{ncr}$ bring further clarity to the ecology and evolution as well as taxonomy of *Symbiodinium* clade types. Recently, Hume *et al* (2015) have identified a new symbiont species (i.e. *Symbiodinium thermophilum*) in the Persian Gulf using $psbA^{ncr}$ that has been identified as C3 type using ITS2 genetic marker. This new species has been identified as being a monophyletic *Symbiodinium* specific to the Persian Gulf (hottest sea in the world, 35°C) and different from recorded outside Persian Gulf (i.e. Gulf of Oman) (Hume *et al.*, 2015). Consequently, the resolution of this genetic marker must be considered in our study and using different genetic marker than ITS2 should be considered in further studies to improve taxonomy resolution.

Secondly, phenotypic plasticity (i.e. physiological characterization) of symbionts need to be considered which can broaden the niche for the symbiont to populate different thermal regimes and therefore broaden the distribution of specific coral holobionts. The change in cell density and chlorophyll concentration (Fitt *et al.*, 2001; Ziegler *et al.*, 2014), biochemical composition and cell volume (Hoadley *et al.*, 2015, 2016), efficiency of photosystem II (Smith *et al.*, 2005) are known factors determining symbiont susceptibility to environmental stressors. Importantly, persistence of coral populations under natural long term thermal exposure (e.g. Arabian Gulf) improves their thermal tolerance despite their symbiotic partner genotype. For example, Howells *et al* (2016) found that *P. daedalea* living in the Arabian Gulf harbored less tolerant C3 symbiont clade and exhibited more thermal tolerance than the same species living in Gulf of Oman and harbor thermal tolerant D1 clade. Levin *et al* (2016) confirmed this pattern and found that C1 *Symbiodinium* living in higher thermal regimes (as per Howells *et al.*, 2012) upregulated ROS scavenger and chaperone genes without stress signs, in comparison to C1 that inhabit lower thermal regimes. Therefore, the phenotypic acclimation of the symbiont can determine thermal susceptibility of corals, and not only its genetic variants (see also, Tchernov *et al.*, 2004), should be considered in construction of bleaching models for accurate predictions (see van Woesik *et al.*, 2010).

Despite the observed conserved *Symbiodinium* community, other components of the holobiont, the microbial community, varied among sites and corals species as previously reported by other researchers (Rohwer & Kelley, 2004; Lee *et al.*, 2012; Carlos *et al.*, 2013; Pantos *et al.*, 2015). It has been suggested that the microbial variations can be influenced by a range of biotic (e.g. host compartment (Sweet *et al.*, 2011a), host physiology (Mouchka *et*

al., 2010), life stage (Sharp *et al.*, 2012)) and abiotic factors (e.g. salinity, depth, temperature, sulfide, nutrients, etc. see; Raina *et al.*, 2009, 2010; Qian *et al.*, 2011; Lee *et al.*, 2015; see also Bourne *et al.*, 2016) that can change microbial composition. As seen also in previous studies (e.g. Rohwer *et al.*, 2002; Littman *et al.*, 2009; Lee *et al.*, 2012; Carlos *et al.*, 2013; van de Water *et al.*, 2016), a high proportion of the microbial community was specific to sites and also coral species within this study.

Consequently, the microbial community cannot be treated as a single unit or a whole community. The microbial community should be categorized into 1) ubiquitous and core phylotypes, 2) bacteria filling specific environmental niche, and 3) dynamic microbes responding to changing biotic and abiotic conditions including stressors (Hernandez-Agreda *et al.*, 2016). This categorization of the total microbial community would enable us to identify the importance of each microbial group in dictating host fitness, and would also provide a clearer understanding of the flexibility of the holobiont. Using relative abundance measures to investigate the functional role of the microbial community underestimates the importance of low-abundant phylotypes and is an oversimplified approach to examine for the extremely diverse microbial community associated with corals (Blackall *et al.*, 2015; Bourne *et al.*, 2016; Hernandez-Agreda *et al.*, 2016). For example, in this study >11K OTUs were retrieved during the survey but both knowledge of taxonomy and relative abundance could not provide insight in to the functional role these microbes play in the coral holobiont existing across thermal regimes. Consequently, there is a need to establish better ways to characterize the importance of the microbial community for the success of the coral holobiont.

It is really needed to deepen the current understanding of the microbial community and identify the core phylotypes specific to coral hosts. Recent work by Ainsworth *et al*

(2015) has identified seven phylotypes (in low abundance) (using Fluorescence *in situ* hybridization method) as universal core bacteria associated with corals (i.e. ubiquitous bacteria mainly from *Actinobacteria* and *Ralstonia*) that facilitates host-algal endosymbiosis. Also, the family Endozoicomonacae has gained recent attention as it is found to aggregate within coral host endoderm in close proximity to zooxanthellae symbiont (Bayer *et al.*, 2013b). It would appear that this family of bacteria comprises a relatively high percentage of the coral microbiome across the world (Neave *et al.*, 2016) and it has been assumed to contribute to coral fitness (Bayer *et al.*, 2013a, 2013b). However, the function of Endozoicomonacae has not been fully explored yet, despite it being considered a core element of the coral microbiome (reviewed in Bourne *et al.*, 2016). This highlights the importance of reconsidering the full microbial community regardless of relative abundance so that core phylotypes and their functional role in determining fitness under stressed and non-stressed conditions can be determined.

Recently, it has been suggested that the microbial community can be adapted to local environments by selection of different metabolic genes, particularly photosynthetic I & II genes in oligotrophic waters (Kelly *et al.*, 2014). Zhang *et al* (2015) found that the functional profile was highly driven by environmental variables (phosphate, nitrate, ammonium, nitrite and chlorophyll *a* concentration) where functional gene was related to biogeochemical cycle (i.e. carbon, nitrogen, phosphorus and sulfur, metal homeostasis, organic remediation, antibiotic resistance and secondary metabolism). Furthermore, other work confirmed that functional diversity of corals microbiome is highly different among different habitats and within different coral ecosystem niches (Dinsdale *et al.*, 2008; Tout *et al.*, 2014). These findings are consistent with our results reporting that photosynthetic *Erythrobacter* is specific

to all surveyed sites at the northern Red Sea (chapter 3) and its relative abundance is increased with increasing SST (i.e. southward). Also, the putative function of microbial community under heat stress exhibited different metabolic profiles among sites and treatments (chapter 4). This suggests that the microbial community provides different metabolic services to corals as an adaptive response (at the holobiont level) to extreme environmental conditions in the Red Sea, however this hypothesis needs to be investigated.

Overall, thermal anomalies across the Red Sea appear at higher intensity at the northern Red Sea, however corals remain bleaching free, contradicting global patterns. In addition, experimental investigation into thermal stress (+3 ^{o}C above mean SST) confirmed that the relative tolerance of corals within the northern Red Sea is higher than that of the central Red Sea suggesting that corals existing in the northern Red Sea are tolerant, and in an environment that is below their absolute thermal maximum. The thermal tolerance was not linked to the genetic variability of the symbiotic zooxanthellae assemblage and a high degree of host-symbiont specificity was observed across latitude. Conversely, the composition of non-zooxanthellate consortia was highly variable across sites and coral species. In addition, the shift of the microbial composition coincided with susceptibility to thermal stress suggesting that the plasticity of microbial community may be a key mechanism for coral acclimation in the Red Sea suggesting presence of district microbial phenotypes fill specific environmental niche.

key findings

- The key environmental variables (i.e. SST, Chl-*a* and K_d) varied spatially across latitudinal gradients of the Red Sea. Water temperature declined toward higher latitude (i.e. northward) as expected, while chlorophyll-*a* concentration and K_d were high only in the southern Red Sea. This highlight that the southern Red Sea is not only warmer, but also more productive and turbid than central/northern Red Sea.
- Thermal anomalies contradicted water temperature pattern and were much higher in the northern Red Sea where DHW reached 15.1 °C-weeks in Hurghada, but surprisingly the region remains bleaching free contradicting the global pattern. This rejected the proposed hypothesis and raised questions about to what extent corals in the northern Red Sea are thermally tolerant and what are the acclimation/adaptation mechanisms?
- Investigations of the microbiome associated with key coral species at five sites experiencing different thermal regimes across the northern Red Sea (ca. 1000 km) revealed two patterns; i) Symbiont clade types exhibited a high degree of host-symbiont specificity and, ii) microbial community was extremely diverse and highly variable among sites and corals species. The total microbiomes here suggesting the plasticity of non-zooxanthellate community may be the key mechanism enabling corals to be tolerant to heat stress. This pattern rejected the assumption that coral tolerance in the Red Sea is linked to high prevalence of heat-tolerant symbiont variant.
- Investigation experimentally the capability of corals to survive above local thermal thresholds (+3°C) across different thermal regimes, corals in the northern Red Sea appeared to be more thermal tolerant and maintained higher photochemical efficiency

than the central Red Sea that was more susceptible to heat stress. This pattern and the previous finding where remote sensing data rejected the proposed hypothesis and revealed that the northern Red Sea is more thermally tolerant than central/south Red Sea and suggests it possibly may be a coral refugia.

- However, the symbiont clade type was same at both northern and central Red Sea, the tolerance of corals at northern Red Sea coincided with stability in microbial structure and diversity, while heat stress influenced microbial community with a signature of trophic mode shift. This suggest presence of specific microbial phylotypes fill specific thermal niche at the northern Red Sea which accept the assumed hypothesis.

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6. *Reference*

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Appendix

Appendix

Table **S1**. Frequency of bleached coral genera recoded during mass bleaching events along Red Sea indicated by (+) for each event. Highlighted fields indicated most frequent genera in all bleaching events

No.	Coral genera	1998	2007	2010	2012	Total
1	Acanthastrea	+		+		2
2	Acropora	+	+	+	+	4
3	Astreopora			+		1
4	Coeloseris			+		1
5	Coscinarea			+		1
6	Ctenactis			+		1
7	Cyphastrea			+		1
8	Diploastrea			+		1
9	Echinopora			+		1
10	Favia	+	+	+		3
11	Fungia			+	+	2
12	Galaxea	+		+	+	3
13	Gardineroseris	+		+		2
14	Goniasatrea	+		+		2
15	Goniopora			+		1
16	Herpolitha			+		1
17	Heteractis			+		1
18	Hydnophora			+		1
19	Leptastrea	+		+		2
20	Leptoria			+		1
21	Leptoseris	+		+		2
22	Lobophyllia	+		+		2
23	Lobophyton			+		1
24	Merulina	+		+		2
25	Millepora	+	+	+	+	4
26	Montastrea			+		1
27	Montipora	+		+	+	3
28	Mycedium			+		1
29	Oulophyllia			+		1
30	Pachyseris	+		+		2
31	Palythoa			+		1
32	Pavona	+		+		2
33	Platygyra			+		1
34	Pocillopora	+	+	+	+	4
35	Podabacia	+		+		2
36	Porites	+	+	+	+	4
37	Psammacora			+		1
38	Seriatopora			+		1
39	Sinularia	+		+	+	3
40	Stylophora	+	+	+	+	4
41	Tubastraea			+		1
42	Turbinaria	+		+		2

Table **S2**. Statistical summary of permutation multivariate analysis of variance (PERMANOVA, permutation level 999) and analysis of similarity (ANOSIM) that performed on microbial community associated with each site (i.e. all corals species within each site) and coral species (i.e. each corals species across sites) separately based on Bray-Curtis dissimilarity matrix. Multifactorial analysis (PERMAONVA) performed to investigate the influence of site and depth on each coral species, while influence of corals species and depth at each site and their interactions on microbial community composition. Both analysis was performed by *adonis* and *anosim* functions in "R" ('vegan' package) with statistical significance level <0.05

Factor / Analysis		**PERMANOVA**						**ANOSIM**	
		DF	Sum of Sqs	Mean Sqs	F Model	R^2	P value	R value	P value
P.nodifera	Depth	1	0.188	0.188	1.321	0.04	0.24		
	Site	4	1.605	0.401	2.814	0.37	0.01 **	0.239	0.01**
	Depth * Site	3	0.921	0.307	2.154	0.21	0.01 **		
F.favus	Depth	1	0.154	0.155	1.18	0.03	0.26		
	Site	4	2.633	0.658	5.037	0.52	0.01 **	0.452	0.001***
	Depth * Site	3	0.368	0.123	0.939	0.07	0.51		
P.damicornis	Depth	1	0.340	0.340	2.501	0.05	0.05 *		
	Site	4	2.729	0.682	5.015	0.47	0.01 **	0.432	0.001***
	Depth * Site	3	0.545	0.182	1.334	0.09	0.18		
S.hystrix	Depth	1	0.200	0.200	1.123	0.02	0.30		
	Site	4	2.893	0.723	4.058	0.37	0.01 **	0.455	0.001***
	Depth * Site	3	1.580	0.395	2.2164	0.20	0.01 **		
S.trocheliophorum	Depth	1	0.231	0.231	1.4265	0.03	0.20		
	Site	4	3.191	0.798	4.9270	0.46	0.01 **	0.589	0.001***
	Depth * Site	3	1.016	0.254	1.568	0.14	0.06		
X.umbellate	Depth	1	0.529	0.529	2.686	0.09	0.03 *		
	Site	4	2.343	0.586	2.9759	0.40	0.01 **	0.526	0.002***
	Depth * Site	3	0.732	0.244	1.2384	0.12	0.18		
	Microbial community change between coral species + water within each								
		PERMANOVA					**ANOSIM**		
		DF	Sum of Sqs	Mean Sqs	F Model	R^2	P value	R value	P value
Abo Galloum	Depth	1	0.1994	0.199	1.4185	0.02	0.16		
	Coral species	6	4.0558	0.676	4.8089	0.49	0.01 **	0.440	0.001***
	Depth* Species	5	1.0507	0.210	1.4950	0.12	0.05 *		
Ras Mohamed	Depth	1	0.3434	0.343	1.8552	0.04	0.08		
	Coral species	6	3.2172	0.536	2.8967	0.38	0.01 **	0.296	0.001***
	Depth* Species	5	1.9138	0.478	2.5847	0.23	0.01 **		
Abo Galawa	Depth	1	0.1609	0.161	1.3280	0.03	0.28		
	Coral species	6	2.6827	0.447	3.6908	0.44	0.01 **	0.372	0.001***
	Depth* Species	5	0.6353	0.127	1.0488	0.10	0.40		
Meritte	Depth	1	0.3208	0.321	1.6899	0.03	0.10		
	Coral species	6	4.4084	0.735	3.8702	0.44	0.01 **	0.531	0.001***
	Depth* Species	5	1.1690	0.195	1.0262	0.12	0.38		
Wadi El Gemal	Depth	1	1.2056	1.206	7.1137	0.13	0.01 **		
	Coral species	6	3.4578	0.576	3.4005	0.36	0.01 **	0.301	0.001***
	Depth* Species	5	1.6796	0.279	1.6517	0.18	0.01 **		

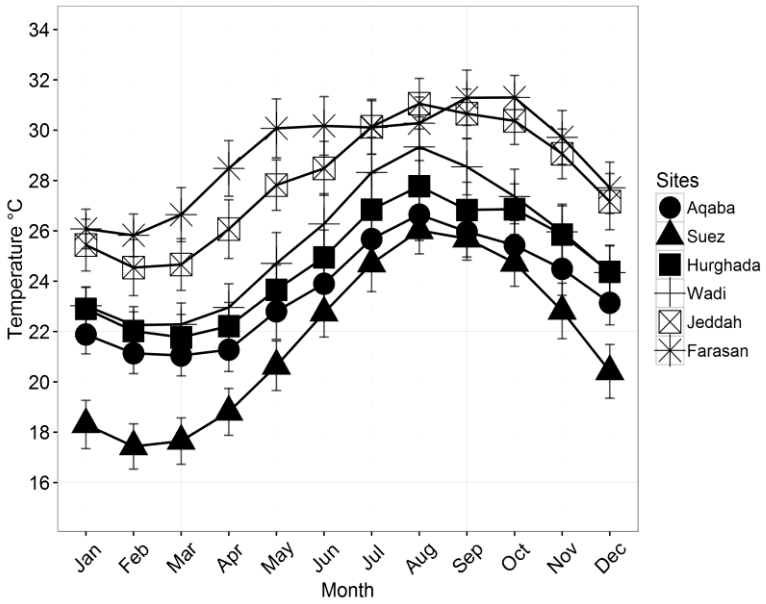

Figure **S1.** Long term mean (Climatology of SST) ±SD in the study sites along Red Sea. Data acquired from remote sensing (CoRTAD - version 5, AVHRR Pathfinder 5.2, 4 km resolution) during last three decades (1982-2012). Climatology calculated as average of each month (n=31) throughout the study period (1982-2012) as stated in Climatology equation in materials and methods.

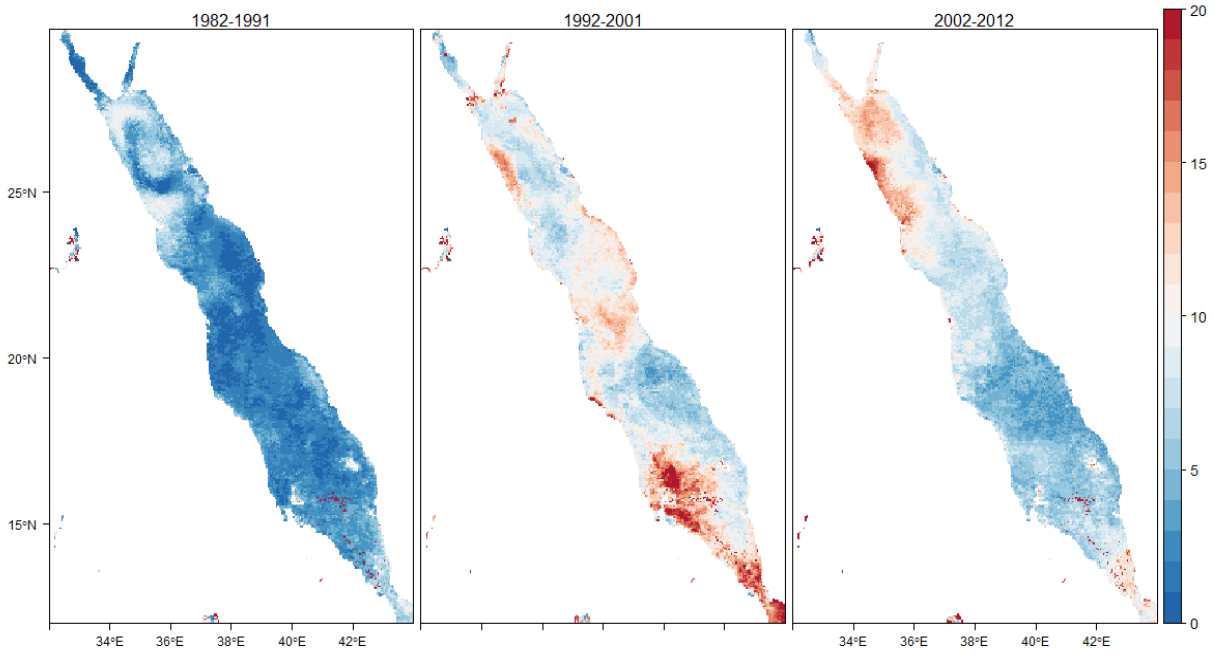

Figure **S2**. Maximum decadal DHW along the Red Sea during the study period (1982-2012). Weekly DHW obtained from CoRTAD-V5 and plotted separately into decades and data showed increasing DHW intensity along the Red Sea in last two decades in both north and south Red Sea, while last decade (2002-2012) showed intensive DHW particularly northern at Egyptian coast.

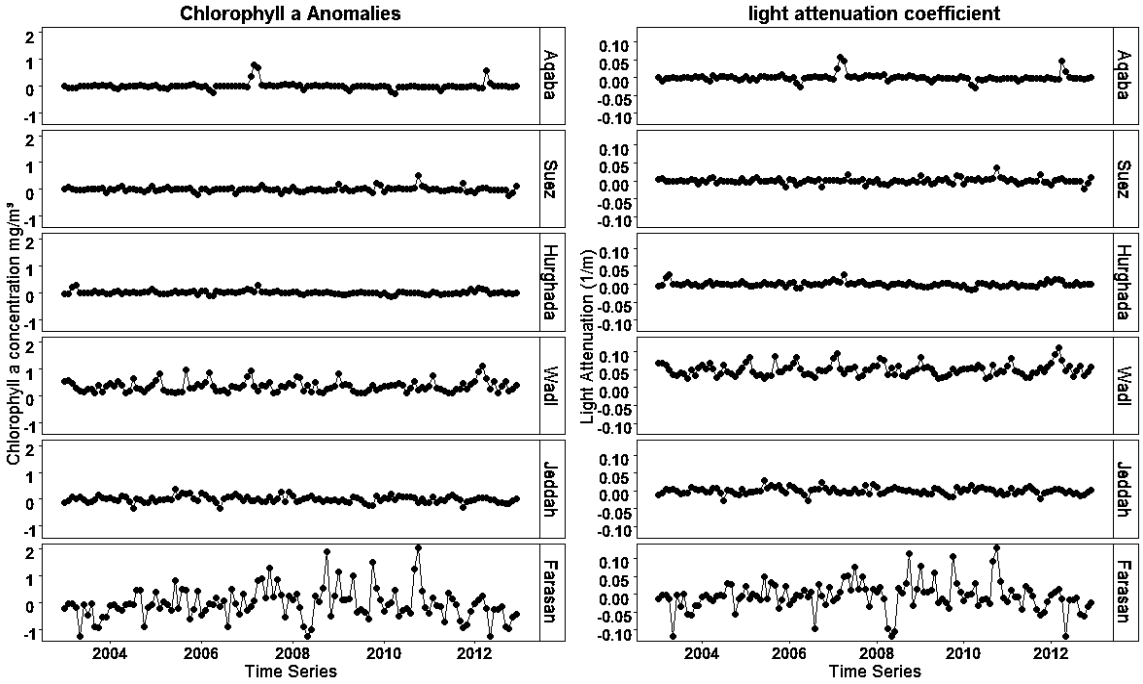

Figure **S3**. Positive and negative anomalies (mutation, n=120) of Chlorophyll a concentration (Chla) and light attenuation coefficient (K_d) (Observations minus monthly long term mean for each site, see; equation in methods section) during the study period (2003-2012). Farasan showed highest (Tukey's test-$p<0.001$) anomalies for both variables (Chla & K_d) in compare to other sites which highlight high fluctuation of both Chla and K_d at the southern Red Sea through water exchange with Gulf of Adan.

Porites nodifera

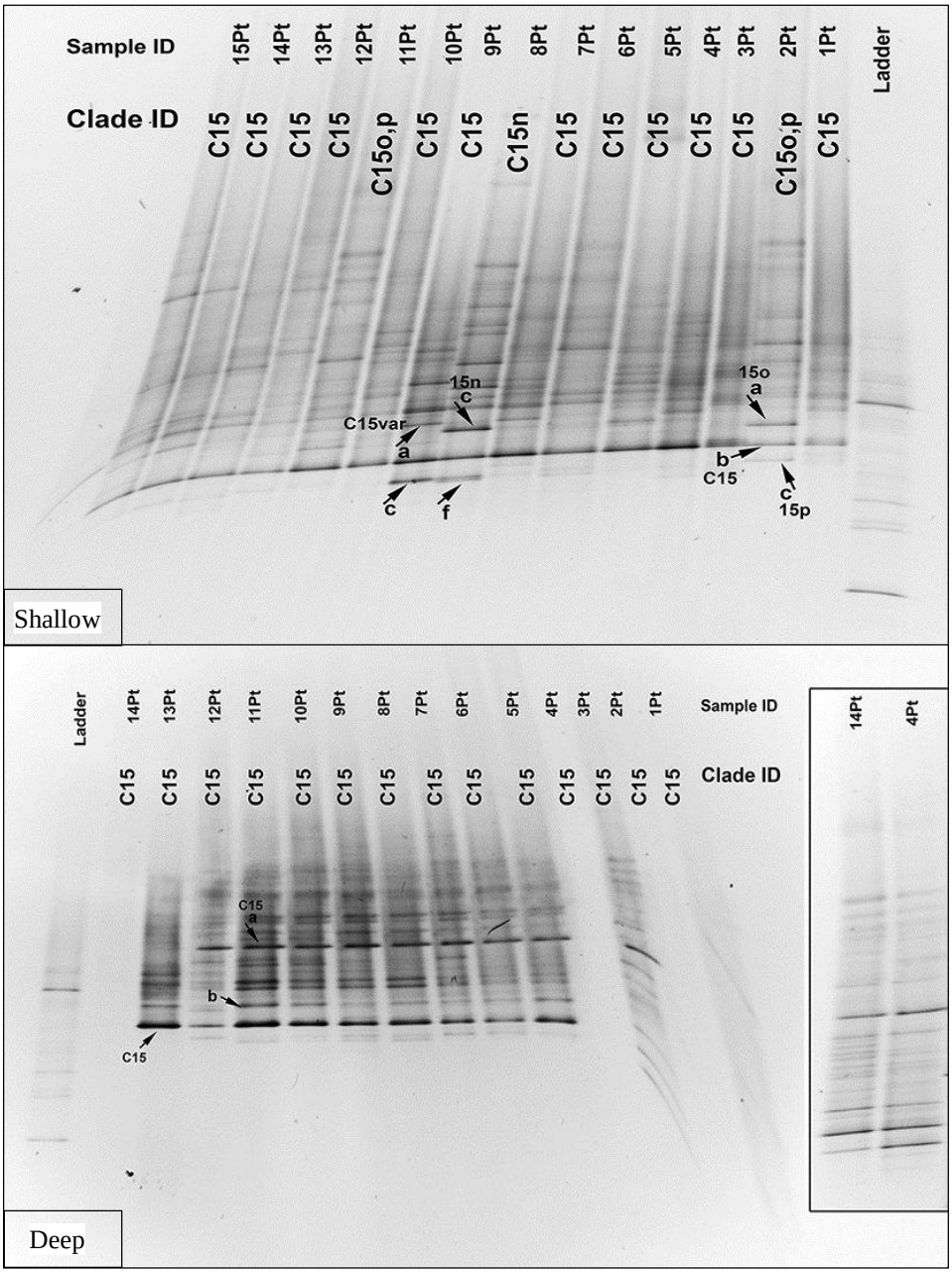

Figure **S4**. PCR-DGGE fingerprints of *Symbiodinium* community hosted within ***P. nodifera*** collected from five sites at two depths (n=15 at each depth) along Egyptian Red Sea latitudes in Febuary-2013. The symbiont type (Clade ID) is given for each lane (sample) where letters indicate lineage type (clade) and numbers indicate ITS2 type (subclade) according to database of Dr. Eugenia Sampayo (Uni of Queensland, AU) and Prof. Todd LaJeunesse (Penn. State University, US). Arrows describe the characteristics bands of the fingerprint and example of heteroduplexes are also indicated.

Favia favus

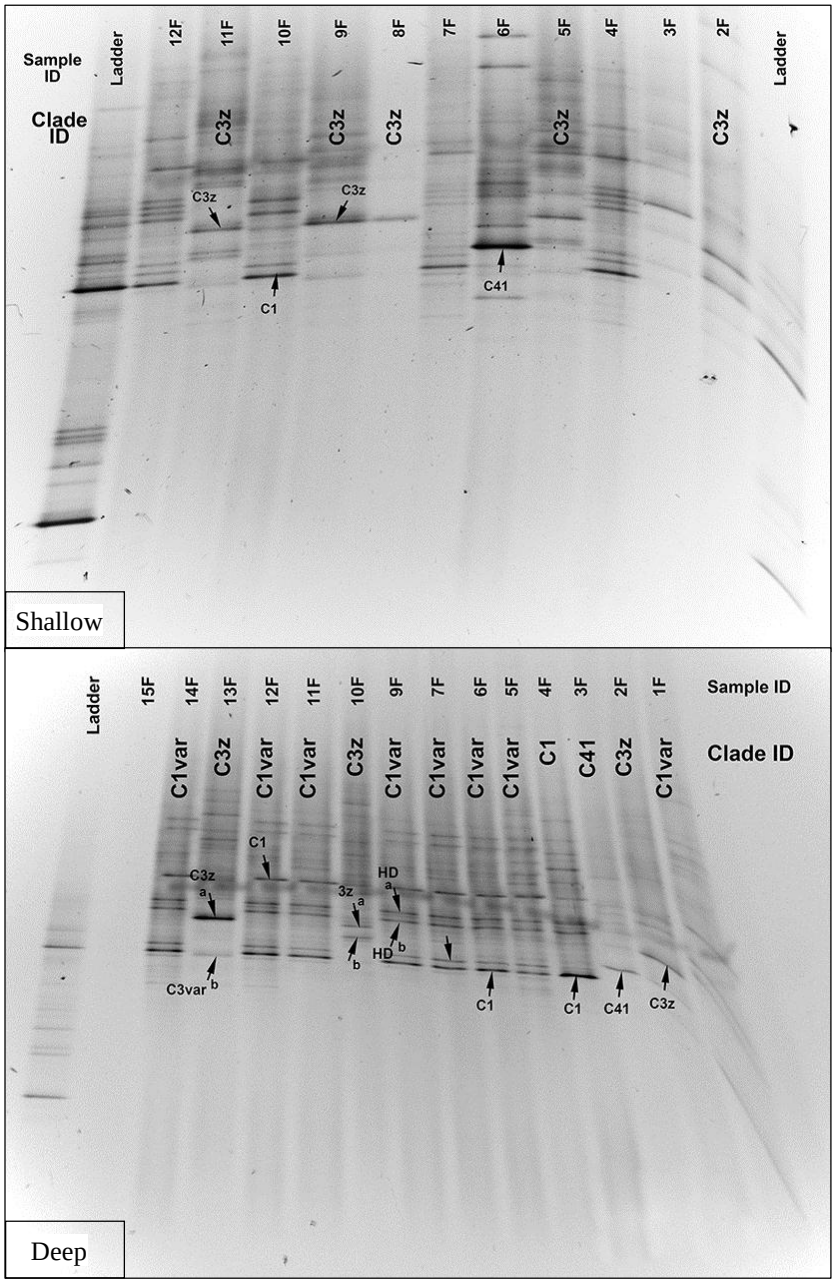

Figure **S5** PCR-DGGE fingerprints of *Symbiodinium* community hosted within ***F. favus*** collected from five sites at two depths along Egyptian Red Sea latitudes in Febuary-2013. The symbiont type (Clade ID) is given for each lane (sample) where letters indicate lineage type (clade) and numbers indicate ITS2 type (subclade) according to database of Dr. Eugenia Sampayo (Uni of Queensland, AU) and Prof. Todd LaJeunesse (Penn. State University, US). Arrows describe the characteristics bands of the fingerprint and example of heteroduplexes are also indicated.

Pocillopora damicornis

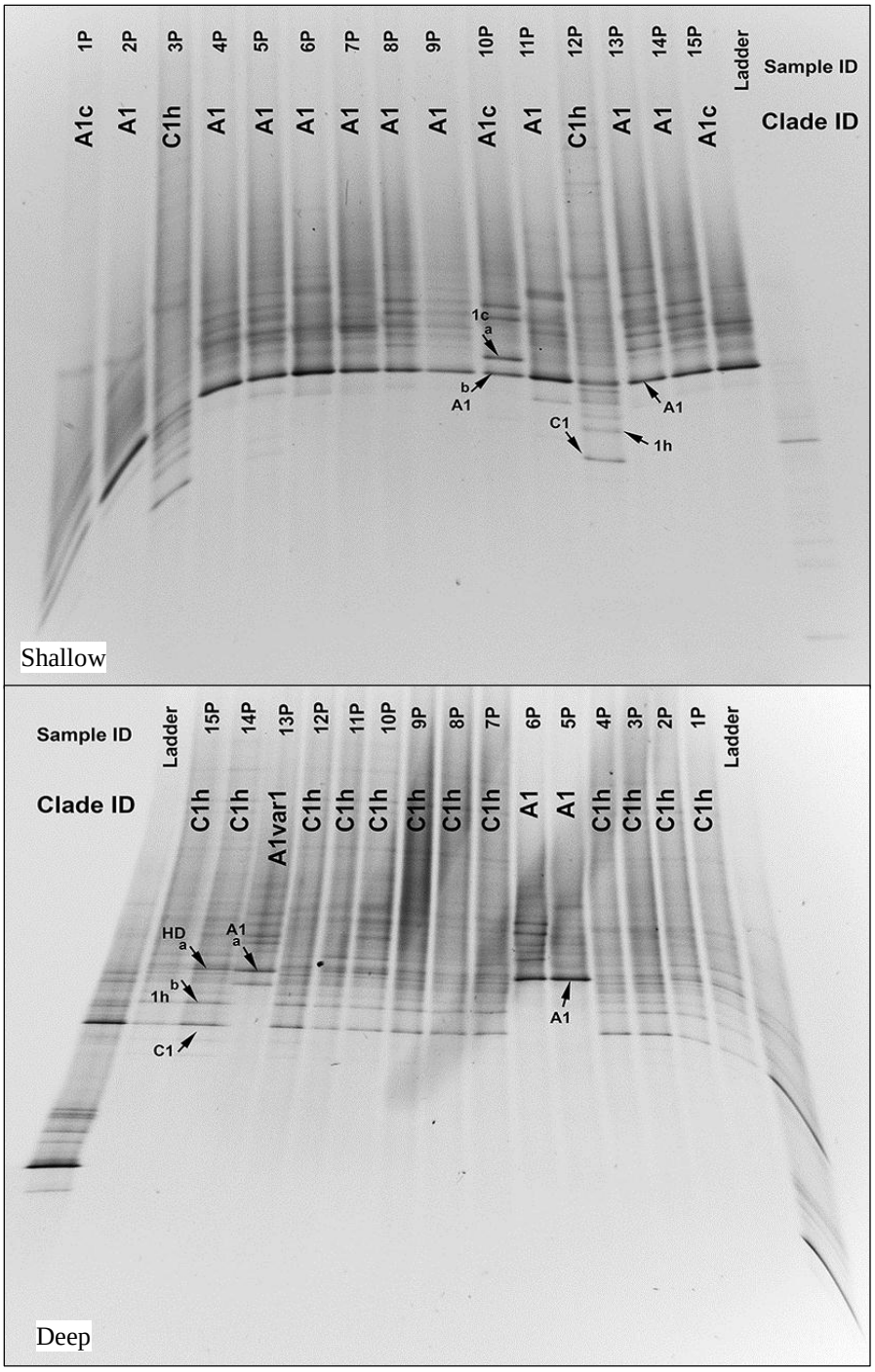

Figure **S6.** PCR-DGGE fingerprints of *Symbiodinium* community hosted within ***P. damicornis*** collected from five sites at two depths along Egyptian Red Sea latitudes in Febuary-2013. The symbiont type (Clade ID) is given for each lane (sample) where letters indicate lineage type (clade) and numbers indicate ITS2 type (subclade) according to database of Dr. Eugenia Sampayo (Uni of Queensland, AU) and Prof. Todd LaJeunesse (Penn. State University, US). Arrows describe the characteristics bands of the fingerprint and example of heteroduplexes are also indicated.

Seriatopora hystrix

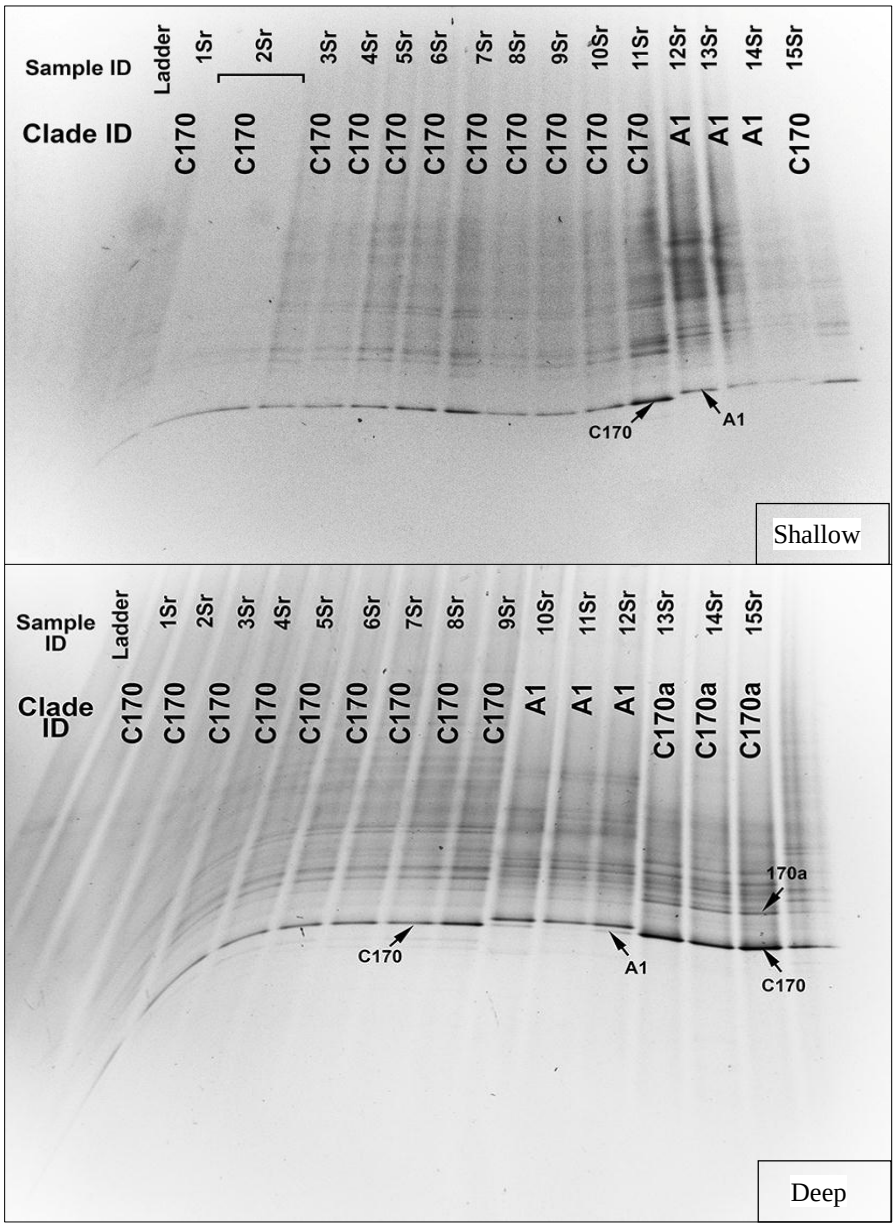

Figure **S7.** PCR-DGGE fingerprints of *Symbiodinium* community hosted within ***S. hystrix*** collected from five sites at two depths along Egyptian Red Sea latitudes in Febuary-2013. The symbiont type (Clade ID) is given for each lane (sample) where letters indicate lineage type (clade) and numbers indicate ITS2 type (subclade) according to database of Dr. Eugenia Sampayo (Uni of Queensland, AU) and Prof. Todd LaJeunesse (Penn. State University, US). Arrows describe the characteristics bands of the fingerprint and example of heteroduplexes are also indicated.

Xenia umbellate

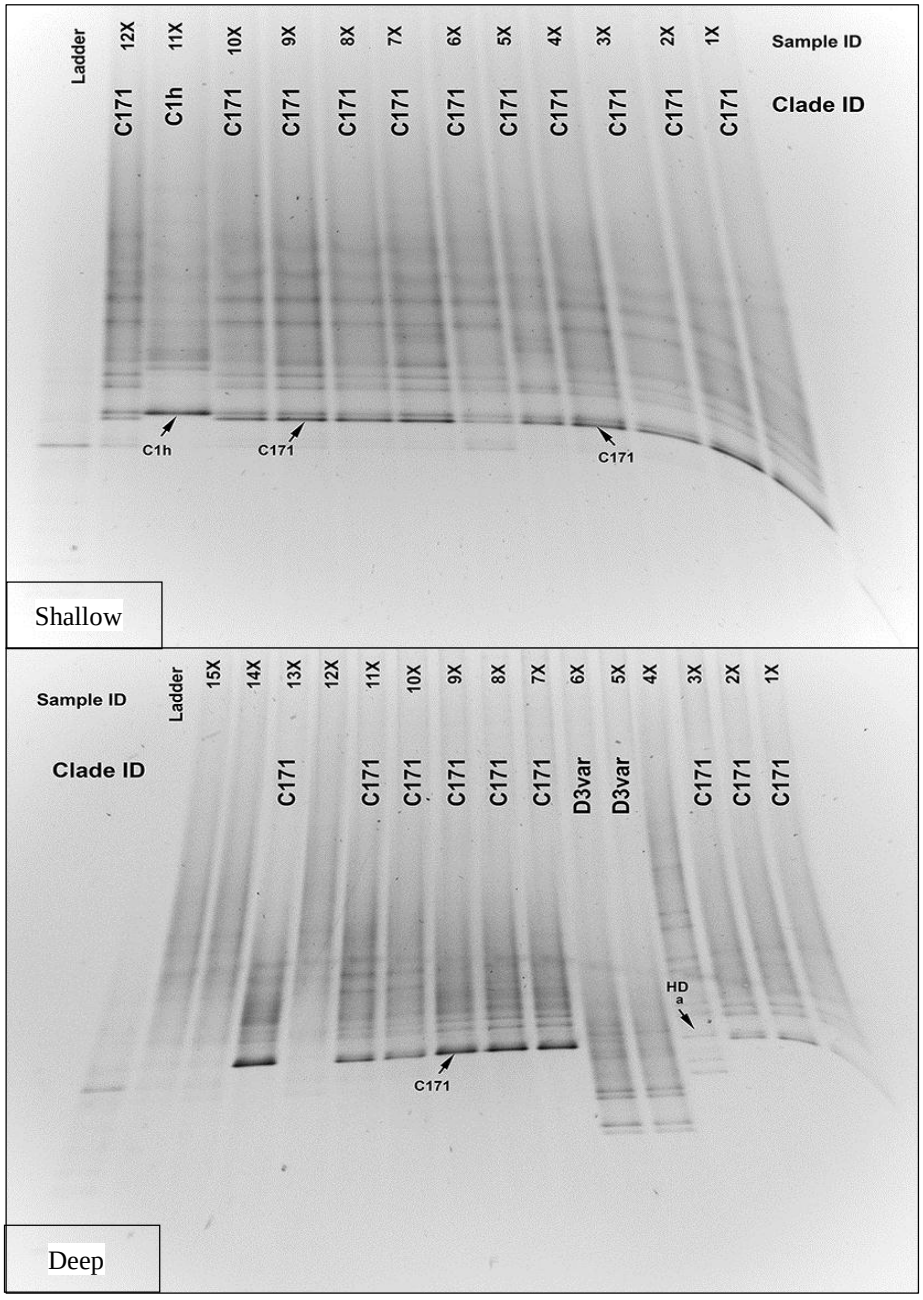

Figure **S8.** PCR-DGGE fingerprints of *Symbiodinium* community hosted within ***X. umbellate*** collected from five sites at two depths along Egyptian Red Sea latitudes in Febuary-2013. The symbiont type (Clade ID) is given for each lane (sample) where letters indicate lineage type (clade) and numbers indicate ITS2 type (subclade) according to database of Dr. Eugenia Sampayo (Uni of Queensland, AU) and Prof. Todd LaJeunesse (Penn. State University, US). Arrows describe the characteristics bands of the fingerprint and example of heteroduplexes are also indicated.

Sarcophyton trocheliophorum

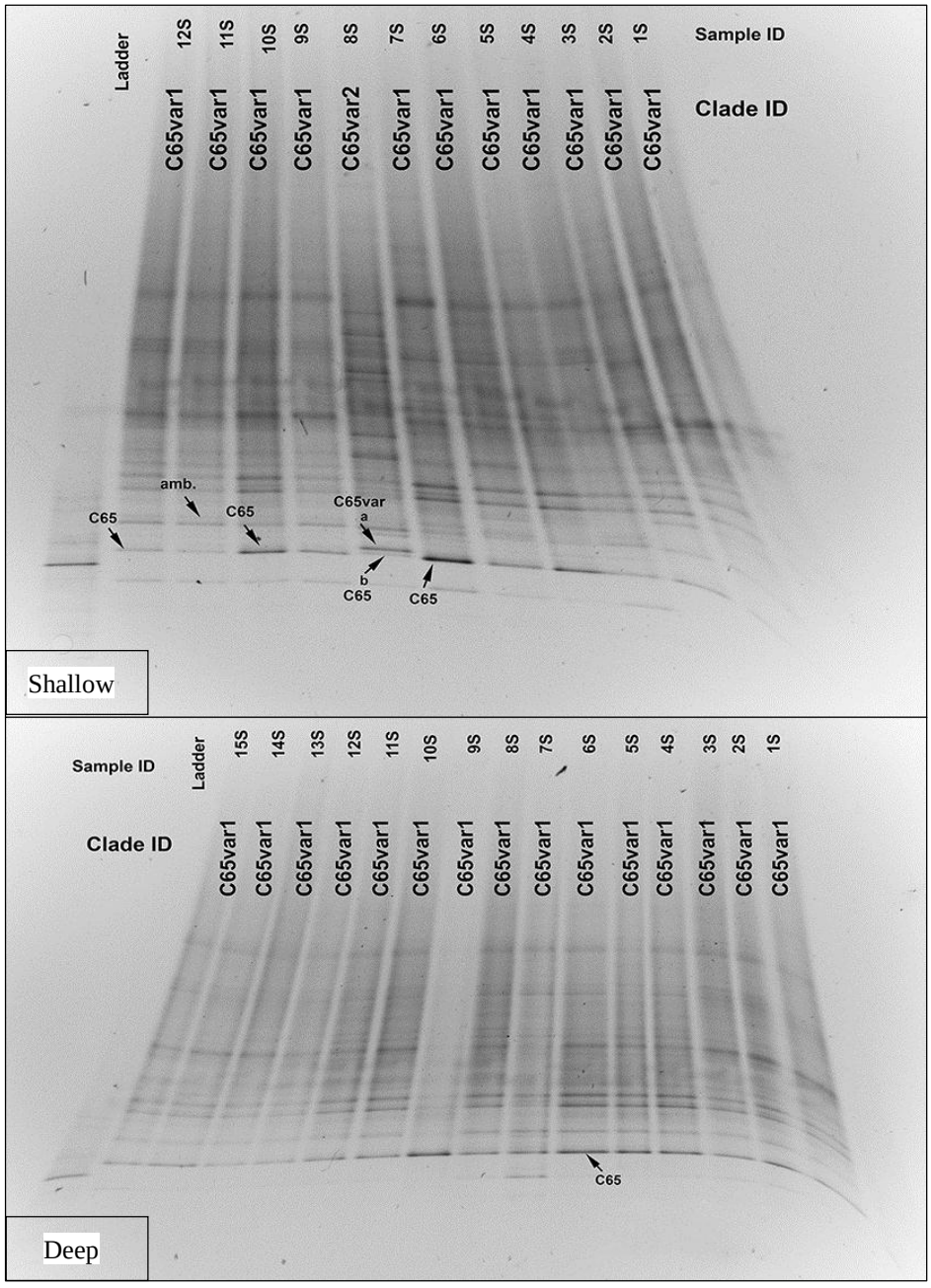

Figure **S9.** PCR-DGGE fingerprints of *Symbiodinium* community hosted within **S. *trocheliophorum*** collected from five sites at two depths along Egyptian Red Sea latitudes in Febuary-2013. The symbiont type (Clade ID) is given for each lane (sample) where letters indicate lineage type (clade) and numbers indicate ITS2 type (subclade) according to database of Dr. Eugenia Sampayo (Uni of Queensland, AU) and Prof. Todd LaJeunesse (Penn. State University, US). Arrows describe the characteristics bands of the fingerprint and example of heteroduplexes are also indicated

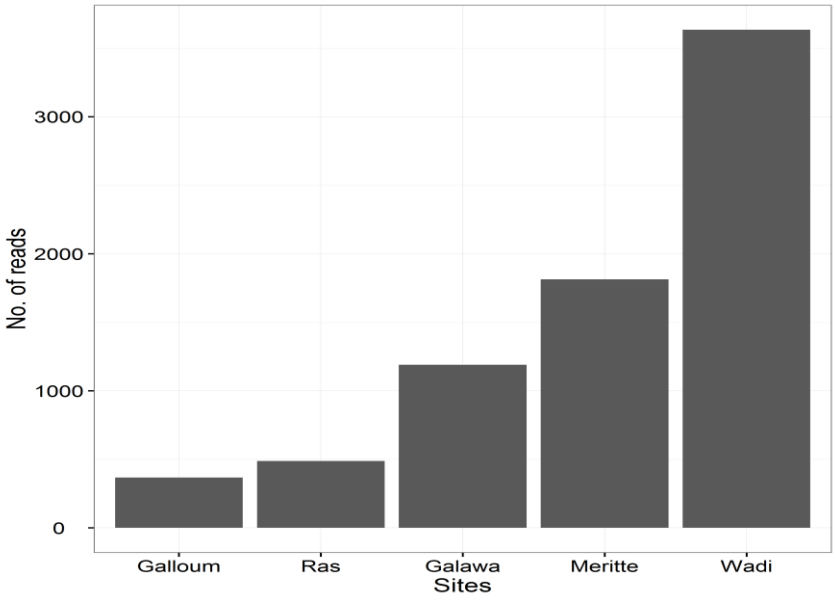

Figure **S10**. Number of OUT reads of site-specific photosynthetic *Erythrobacter sp*. Data obtained from indicator species analysis that performed on total OUT retrieved from surveyed sites. Graph demonstrates increase of number of OTU read from less warm site (Abo Galloum) high warm site (Wadi El Gemal) that increasing southward with increase of SST.

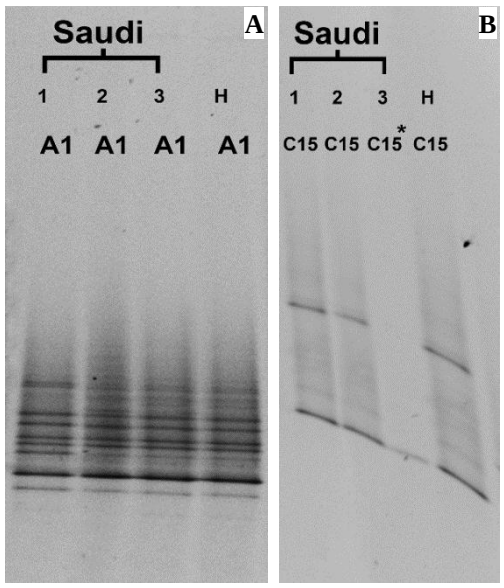

Figure **S11.** PCR-DGGE fingerprints of *Symbiodinium* community hosted within *P. damicornis* (A) and *P. nodifera* (B) collected from both Hurghada and Jeddah in August/November-2013 for short term stress experiment. The fingerprint demonstrates samples collected from Jeddah (n=3) compared/aligned to Hurghada sample (H) has been previously aligned and compared to identified samples during survey. The symbiont type is given for each lane (sample) where letters indicate lineage type (clade) and numbers indicate ITS2 type (subclade) according to database of Dr. Eugenia Sampayo (Uni of Queensland, AU) and Prof. Todd LaJeunesse (Penn. State University, US). Due to missing DGGE profile of *S. trocheliophorum*, its fingerprint is not included

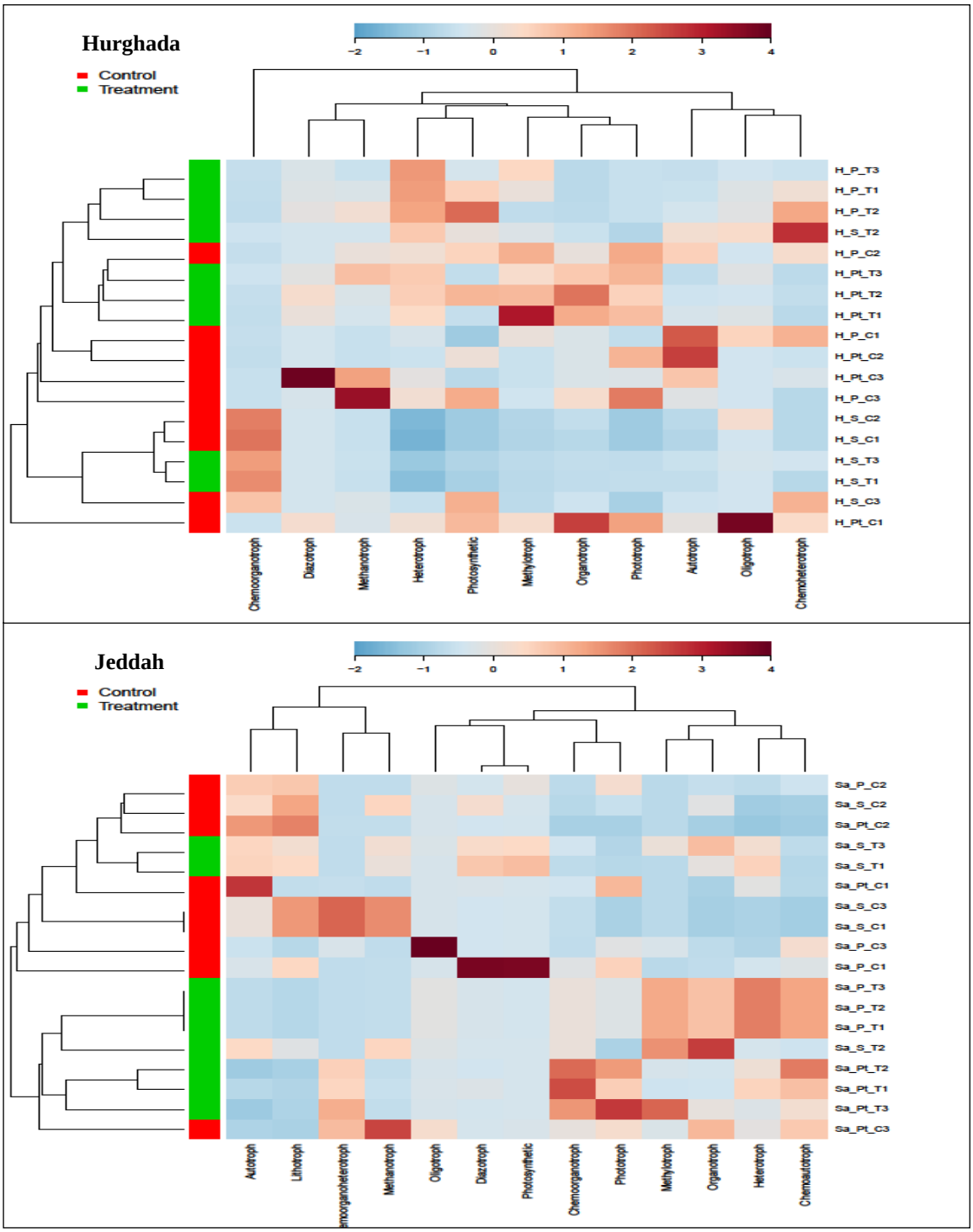

Figure **S12.** Heat maps represents the abundance of trophic mode of microbial community associated with coral species at each site based on the putative taxonomic-to-phenotypic function in METAGENassist web interface after summing of OTUs to genus level. Each heat map represents the difference of trophic mode between control (red) and treated (green) samples and the change are displayed by relative scale (blue to red). Heat map produced using Euclidean distance measure and average clustering algorithm. Samples include Jeddah=Sa and Hurghada=H, and each site include coral species as *P. nodifera*=Pt, *P. damicornis* =P, *S. trocheliophorum* =S, while within each species, samples (n=3) assigned to control=C and Treatment=T