QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

SYMBIOTIC DINOFLAGELLATES ASSOCIATED WITH CORALS IN QATAR: CHARACTERIZATION AND PHYSIOLOGICAL RESPONSE TO ENVIRONMENTAL CHANGE.

BY

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ABSTRACT

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Title: Symbiotic Dinoflagellates Associated with Corals in Qatar: Characterization and Physiological responses to Environmental Change.

Supervisor of Thesis: Dr. Radhouane Ben Hamadou.

Studying coral reefs' responses to environmental and anthropogenic stressors is crucial to ensure the development of appropriate conservation and restoration programs, especially in areas such as the Arabian Gulf where they are subjected to extreme seawater temperature stress and experiencing consistent losses in coverage and distribution. Coral bleaching events witnessed during the last decades in the Arabian Gulf are believed to be the result of the effect of heat events occurring more frequently in the region as a result of the global ocean warming. When corals are under thermal stress, their symbiotic zooxanthellae are affected, and their photosynthetic activity decreases until the stress disappears or until the zooxanthellae are expelled from the coral. To study the response of thermal stress on the zooxanthellae associated with the coral host, I exposed three ecologically important coral species from Qatar, *Porites lutea, Dipsastraea pallida,* and *Acropora downingi*, to three levels of temperature in laboratory aquaria. The three chose species are known to have differential responses to heat events. Response parameters were assessed during and after the experiment using fluorescence technique (Pulse Amplitude Modulation – PAM fluorometry and flow cytometry) to evaluate the coral health by measuring the photosynthetic activity of *Symbiodinium* zooxanthellae. Samples of the three coral species were collected from an offshore reef of Qatar (Um Al-Arshan)

and used to test the effects of different levels of temperature under controlled artificial conditions. Our results suggested that high levels of temperature 35°C affect consistently the photosynthetic activity of zooxanthellae within coral tissue of the three species. *Acropora downingi* was the most sensitive species to the thermal stress. Indeed the measured photosynthetic activity in the 35°C treatment was reduced after 17 days, the coral host bleached after 21 days, and all the colonies died after 24 days. The photosynthetic activity of *Dipsastraea pallida* was overall lower than the other studied species. At 35°C, it started to decrease after 17 days, but *D. pallida* showed the less decrease in photosynthetic activity throughout the experiment. Finally, *Porites lutea* showed the highest decrease in photosynthetic activity at 35°C. No decrease in photosynthetic activity was detected at 30°C for the three species. The enumeration and physiological characterization of zooxanthellae extracted from the corals subjected to three different temperature treatment (25ºC, 30ºC and 35ºC) were conducted using both flow cytometry and investigation under microscope. Results showed a consistent reduction of the ratio healthy/unhealthy cells among all three considered species when temperature was increased.

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CHAPTER 1: INTRODUCTION

1.1 Coral reefs and their importance

Corals are the ecosystem engineers of coral reefs. They have very slow growth rate, about 1 cm/year and some even grow only about 3-20 mm/year (Lough *et al.*, 2002). Hence, some reefs were produced over many million years (Veron, 2000). As these corals grow and die, their limestone skeletons remain, offering bare substrate for other corals to settle on and grow (Lough *et al.*, 2002). Throughout the years, these corals are able to form massive and intricate three-dimensional calcium carbonate structures. Corals are very important in shaping the reefs and providing habitats to associated fauna and flora, and provide important ecosystem services to humans (Hallock P., 2001; Moberg and Folke, 1999). Coral reefs contribute of one quarter of the total marine biodiversity, although they only count of 0.1% of the ocean floor surface. The amazing diversity of invertebrates and fish species found in coral reefs is often compared to that of vertebrates and arthropods in tropical rainforests. Many billions of humans are dependent on the services and goods provided by coral reef ecosystems. Coral reef ecosystems are very productive and provide us with services such as supporting, regulating, and provisioning services. Indeed, they protect seashores from waves and storms. Finally, they contribute to carbon and nitrogen fixing, and play a role in nutrient recycling (Rädecker *et al*., 2015)

1.2 Threats to coral reefs

Even with their huge biological, economical and attractive values, it is estimated that 20% of the world's coral reefs have been damaged mainly by anthropogenic activities (Miththapala, 2008). Remaining reefs are under threat of collapse mainly because of anthropogenic activities. It has been estimated, if these activities and destruction persist,

that 70% of the coral reefs worldwide will be damaged by 2050 (Johnson *et al*., 2003). Human-based activities or anthropogenic environmental stresses are the main and significant threats to the coral reefs such as climate change, marine pollution, overexploitation and overfishing. With the increase in human activities, such as industrialization, urbanization and burning of fossil fuels, the level of atmospheric carbon dioxide has continuously increased, which has led to global warming of both aquatic and terrestrial ecosystems (Vitousek, 1994). It has been estimated the atmospheric carbon dioxide is increased by 30% since the preindustrial period (Calderia *et.al*, 2003). The more anthropogenic carbon dioxide pumped into the atmosphere, the more will by absorb by the oceans (as a sink) which will trap more heat and increase the temp of the ocean (Calderia *et.al*, 2003). Additionally, the increase in use of chemicals coupled with poor land management has also brought pollutants to the ocean through water flow (Browne *et al.,* 2015). The resulting changes in oceanic ecosystems due to the above-mentioned pressures have triggered the degradation of coral reefs in many parts of the world ocean such as Southern Florida, the Caribbean, and the Arabian Gulf (Sheppard *et al*., 2010; 2012; Browne *et al.,* 2015; Sheppard, 2016; Shuail *et al*., 2016). The increase of the human population has resulted in over-fishing. Large fish are disappearing, and fishing activities of other large crustaceans such as shrimps have led to the damage of coastal areas (Jackson *et al.,* 2001). Both over-fishing and overexploitation of different resources disrupt the balance of coral reefs and affect the biodiversity and stability of the ecosystem.

Human development has affected the coral reef ecosystem by disrupting the marine environment. Increased activities of poor agriculture and other land-based activities have

led to rises of freshwater runoff, causing huge amounts of sediments and nutrients to be discharged to the coastal waters, both detrimental to coral reefs. Indeed, these sediments contain huge amounts of nutrients which increase the turbidity of the water, promote the growth of algae detrimental to corals, and can cause eutrophication. Changes of nutrient amounts, lead the over growth of other organisms such as algae that can disrupt coral reef ecosystem. Eventually, turbidity and eutrophication damage the coral reefs by limiting the amount of sunlight that reaches the corals. As mentioned above, hard corals need sunlight for photosynthetic zooxanthellae for providing the corals with nutrients. Corals have to survive on their nutritive reserves if their zooxanthellae are expelled from their tissue and they bleach. In cases of extended stress, bleached corals will eventually die (Wooldridge, 2013).

1.3 Global warming and climate change and their threats to coral reefs

Global warming and subsequent changing of climate are causing severe threats to coral reefs that are already under other anthropogenic stresses. The warmer atmosphere and sea surface temperature caused by global warming affect corals and disrupt coral reef organisms by causing coral bleaching and changing ocean chemistry. It has been estimated that seawater temperature at Gulf area in July and August reached 32-34 °C (Coles and Riegl, 2013). This affects not only corals, but other communities that use coral reefs as home or habitat.

1.4 Coral anatomy and physiology

The framework of coral reefs is formed by reef-building coral colonies, which thrive in nutrient-deprived waters and inhabit warm waters close to their upper thermal limits

(Muscatine and Porter, 1977; Wild *et al*., 2011). Corals are marine invertebrates belonging to a large group of sessile animals called Cnidarians. They are mostly colonial and are composed of up to thousands of polyps (Gates and Edmunds, 1999). Each polyp is like a bag full with fluids attached to a ring of tentacles around it mouth (Figure 1).

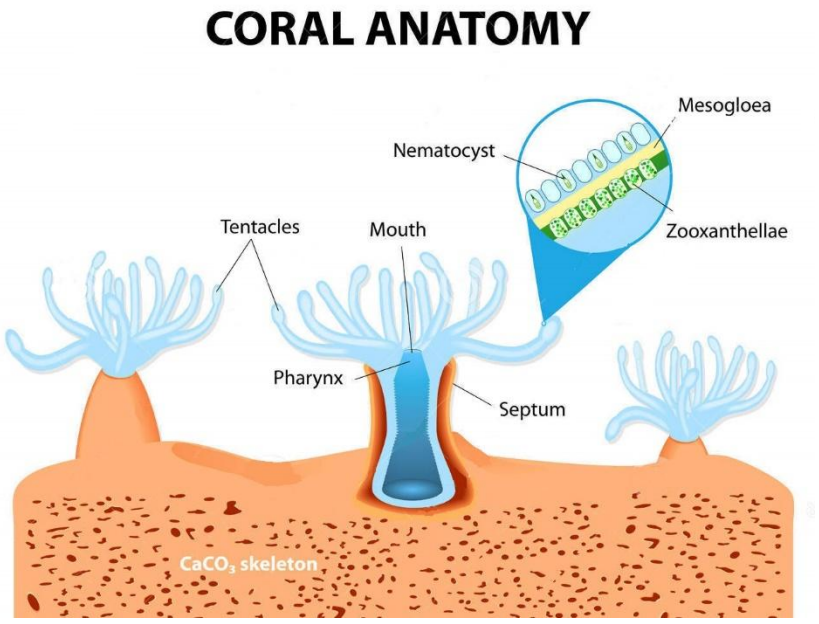


Figure 1: Anatomy of a coral polyp depicting the general constituents and the inner structure of a tentacle with nematocyst and zooxanthellae (retrieved from dreamstime.com on April 12, 2017)

Polyps within a colony are connected by living tissue and can share and exchange food among them (Miththapala, 2008). All hard corals are reef-building, and each polyp produces a protecting skeleton by extracting calcium carbonate from the sea water and depositing it at its base to form a calcium carbonate "cup" called a corallite. Reef building organisms are able to build reefs by secreting hard calcareous material for their skeletons, to form the hard stony base of the reef (Goreau, 2006). This limestone

(calcium carbonate) skeleton provides shelter for the polyps, which are able to extend out of, or fully retract into their corallites. Polyps have a gastro-vascular cavity that play the role of stomach for the polyp (Saxena, 2015). The tentacles that are surrounding the mouth of the polyps contain nematocysts and are used for both for protection and to capture small animals for food such as zooplankton (NOAA, 2007).

1.5 Zooxanthellae and Corals:

Most scleractinian corals are hermatypic and live in symbiosis with dinoflagellates from the genus *Symbiodinium,* hosted in the coral tissue, also commonly known as "zooxanthellae". Zooxanthellae are unicellular photosynthetic organisms; require light and nutrients (Sumich, 1996). They are prominent on the coral tissue ($1.5\ 10^6$ zooxanthellae per cm^2) and each zooxanthellae has 2-10 pg of chlorophyll. The coral-zooxanthellae relationship is addressed around the exchange of the nutrition, zooxanthellae transfer most of their fixed carbon comes from the photosynthesis process to the coral and return coral provide them with inorganic nitrogen, carbon dioxide, phosphorus, as well as shelter to protect them and environment with light source (Baker, 2003). Therefore, corals that contain zooxanthellae are distributed mostly in the shallow photic zone, but some species can also be found in deeper waters, into the mesophotic zone in the western areas of the Indian Ocean, Pacific Ocean and Greater Caribbean (Brifge *et al*., 2011). There are many described and undescribed species of zooxanthellae that associate with corals, with different coral host species associating with different symbionts, with some geographical variability (Baker, 2003; Hume, 2015). It is not well

known how the *Symbiodinium* community is selected, but some molecular clades are known to have a higher tolerance to thermal stress (e.g., Hume, 2015).

The zooxanthellae live in vacuoles within cells in the inner layer of the coral tissue (Yellowlees et al., 2008). This symbiosis interaction involve the ability of the zooxanthellae to colonize the host coral , the capability of the coral host to tolerate the existence of the zooxanthellae and adaptation mechanics for exchanges the nutrients (Douglas, 1994). Zooxanthellae in good light environment of clear reef waters can display great rate of photosynthesis, which produce huge quantities of dissolved oxygen. Reactive oxygen species can results from the high concentrations of oxygen (Weis, 2010). Cellular damage can happened because of the Reactive oxygen species through oxidizing membranes, denaturing proteins and destruction of nucleic acids (Weis, 2010). Both coral and zooxanthellae have adaptation mechanics for handling the reactive oxygen species to avoid cellular damage by expressing managing enzymes such as catalase and superoxide dismutase (Richier et al., 2005). These enzymes can convert the reactive oxygen back to oxygen and water (Weis, 2010). Disruption of the coral-zooxanthellae symbiosis cause coral bleaching where the zooxanthellae are expelled from the coral tissue (Douglas, 2003). Bleaching cascade happened when the reactive oxygen species managing responses increased during stress in way they can't handle it.

1.6 Coral bleaching

Coral bleaching is a phenomenon during which the coral-zooxanthellae symbiosis is disrupted and the zooxanthellae are expelled from the host coral's along with their photosynthetic pigments (Krediet, 2013). Coral bleaching described the change of physical appearance of corals that appear to be white due to the skeleton becoming

visible through the transparent coral's tissue (Fitt *et al*., 2002). This loss can be caused by several environmental stressors such as high seawater temperature, ocean acidification and change in the available light for corals (Douglas, 2003 and Baker *et al*., 2008). Coral bleaching can cause coral mortality if the environmental stress still present (Wilkinson, 1998 and Baker *et al*., 2008). The cell density of symbiotic zooxanthellae is almost 1.5 10^6 zooxanthellae per cm^2 of the surface of coral and is influenced by environmental disruption such as temperature and amount of light. Many important coral mortality events have been observed in the last 20 years after the global coral bleaching events. High mortality rate noticed in the southeastern Arabian Gulf mainly in UAE and Qatar and recovery after almost a decade was only showed in small areas (SCENR, 2007).

.Corals face different natural threats such as strong waves that can easily break corals to fragment or high seawater temperature that cause the death of photosynthetic live in corals. These extreme environmental stresses can have disturbing consequences on a coral's physiology when not leading to their death. Coral reefs can recover from natural threats or other environmental stresses caused by anthropogenic sources, but if they are under recurrent exposure or long period exposure, this can affect their tolerance to stresses and reduce the possibility of recovery to healthy status and they eventually will die (NOAA, 2007).

1.7 Methods to analyzing the zooxanthellae response to thermal stress

1.7.1 Pulse-Amplitude-Modulation flurometer

The symbiont photosynthesis is critical for the coral survival as it provide the coral with the vital photosynthetic product. Many factors control the photosynthesis process of the zooxanthellae associated with coral such as different molecular clades of zooxanthellae

that exhibit different photosynthetic efficiency. Chlorophyll fluorescence measurements is very powerful tool to observe the physiological response of the zooxanthellae associated with coral (Fitt et al., 2001). Many analyzing features was discovered by the chlorophyll fluorescence measurements leading to more understanding of the complexity of photosynthesis process under different environmental condition .with the increase of research in this filed, an instrument was developed called Diving PAM Pulse-Amplitude-Modulation flurometer to assess *in situ* photosynthesis process by measuring he maximal optimal quantum yield (*Fv/Fm*) of photosystem II of the zooxanthellae. The saturation pulse prompts maximal fluorescence yield, Fm, and maximal variable fluorescence, Fv=(Fm-Fo) (Salih. *et.al*, 2006). In addition to the maximum quantum yield, PAM instrument can process many analysis of the photosynthesis

1.7.2 Flow cytometry

Recent studies have been used new methods to assess the zooxanthellae response to environmental stress such as thermal stress by measuring the fluorescence of photosynthetic pigments (Lee *et al*, 2012). Flow cytometry has been used to quantify the zooxanthellae cells and statue the health of each cells as a stream of particles pass through the instrument, each cell pass through laser illumination to allow measuring both light scattering, related to cell size and complexity or shape (Krediet *et al*, 2015). This methods showed to be effective to analyze large number of the cells in short time.

1.8 Status of the corals in the Arabian Gulf

The Arabian Gulf is a semi-enclosed, very shallow sea with average depth of 35m; limited water exchanges with adjacent basins and high evaporation rates that result in sea temperature ranges of 21 to 34°C and salinity of 37 to 44 psu and combination of these

elements result in disfavoring conditions for coral growth (Taher *et al*., 2012). The photic zone of the Arabian Gulf which is sunlight zone commonly reaches 6-15 meter only due to the basin's high turbidity (Siebold, 1973). The limitation of coral reefs in the Arabian Gulf is due to extreme temperature range which has also increased in the last 10-15 years (Taher *et al*., 2012). In addition to temperature, the Arabian Gulf shows high salinity due to high evaporation rate, which go beyond the rainfall and freshwater inputs (Sheppard, 1993). It has been reported that many areas in the Arabian Gulf are covered with poorly developed reefs dominated with *Porites* and *Acropora* (Sheppard, 2012). Reef-building corals (Cnidaria: Anthozoa: Hexacorallia: Scleractinia) are mostly found on the northern and eastern coasts of Qatar, where the environmental conditions are best for coral growth (Rezai et al. 2004). Although the total number of species recorded in the Arabian Gulf reaches 66 species (DiBattista et al. 2016), few of them have been documented to occur in Qatar, possibly due to the sparse work conducted in the region (Rezai et al. 2004).

Coral communities vary in composition from mono-specific species mainly *Porites* and *Acropora* sp. to more diverse communities made up of massive *Dipsastraea* colonies. In the Gulf region, 50 species of hard corals and approximately 200 species of reef-associated fishes are supported by coral reefs (Pilcher *et al*., 2000). Reefs in the Arabian Gulf cover by 33% of live coral during 1992 and 1994's surveys, and presented a slight decrease to 31% by 1999 (Pilcher *et al*., 2000).

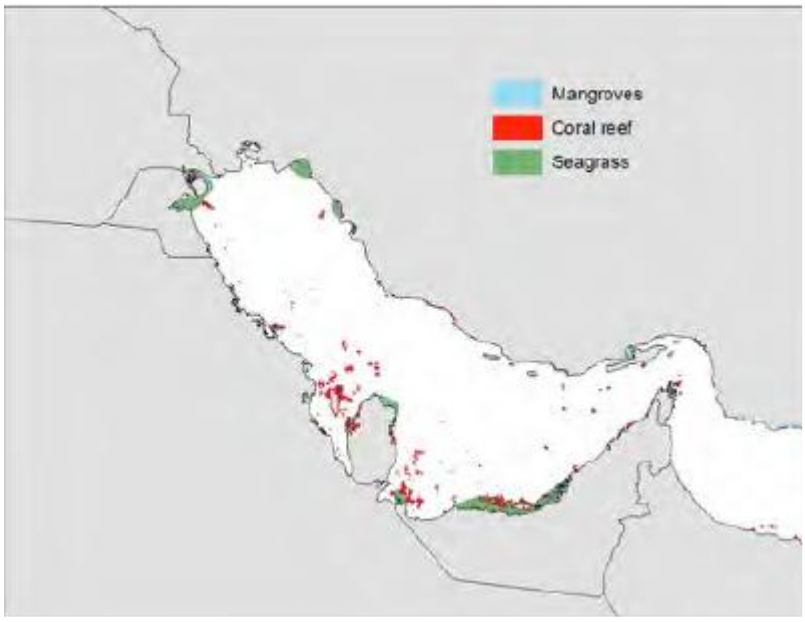


Figure 2: coral reefs distribution and other marine ecosystems in the Arabian Gulf (Retrieved from: AGEDI. 2015. Technical Report: Regional Marine Biodiversity Vulnerability and Climate Change)

The Arabian Gulf reefs persisted the effects of oil spills associated with the Gulf War, however they were affected by high seawater temperature that reached more than 34ºC in summer 1998 (Vogt and Al Shaikh, 2000). Qatar has witnessed an exponential decrease in coral, which has been apparent throughout the Gulf region in recent years (Burt *et al.,* 2016). Also a considerable high mortality rate was noticed in the southeastern Arabian Gulf mainly in UAE and Qatar, and recovery after almost a decade was only showed in small areas. (SCENR, 2007).

With the combination of the above stress, the corals in the Arabian Gulf show a high adaptability to the extreme environment they live in. Compared to other regions in the world, corals in the Arabian Gulf have an extremely high temperature tolerance. Indeed, when exposed to temperatures above 33°C, the survival rate of corals from the Arabian

Gulf was higher than those from the Sea of Oman (Howells *et al.*, 2016). Indeed, most corals in the Arabian Gulf are exposed to sea water temperatures of 35°C in the summer, and are able to survive year after year. The high thermal tolerance is thought to be due to their symbiotic zooxanthellae community. For example, the symbiont *Symbiodinium thermophilum* is known to have a high tolerance to high temperature, and has only been found in the Arabian Gulf (Hume et al., 2015)

CHAPTER 2: LITERATURE REVIEW

The symbiotic relationship between zooxanthellae and their coral host can be altered with environmental stresses such as increased seawater temperature. Methodologies have been developed to study the response of corals to different temperature levels. Studies related to the status of the coral reefs have increased recently especially in areas such as the Arabian Gulf whose corals are facing extremely high temperature (Coles and Riegl, 2013). The ability of coral to survival and tolerate the thermal stress is related to their ability to associate with types of the *Symbiodinium* (Howells et.al. 2016). Zooxanthellae species are distributed among nine molecular clades (Clades A-I) and only (clades A-D) are associated with coral host and they are differ in their physiological response and tolerance ranges to temperature (Stat. *et al.*, 2008). However, most coral species are host only single type of the zooxanthellae, still there are some coral can also host several types of the zooxanthellae (Howells *et al*, 2013). New studies have showed that most of coral species on the Great Barrier Reef are associated mainly with one type with another type present at low level (Ulstrup & van Oppen, 2003). Many combination of the coral and the zooxanthellae type may give ecological benefits in many ecological places, with novel evidence that some zooxanthellae type can influence the growth of coral and their ability to tolerate and overcome different environmental stresses (Howells *et al*, 2013).

Different coral species respond differently to thermal stress. For example *Porites* species are known to be tolerant, *Acropora* species are known to be sensitive, and *Dipsastraea* species are known to have intermediate tolerance sensitivity (Howells *et al*, 2011 and

Furby *et al*, 2013).

Characterizing zooxanthellae is useful to identify the zooxanthellae community present and to identify the potentially tolerant clades that are resistant to thermal stress. Zooxanthellae species with molecular clades (Clades A-D) that are associated with coral host have potentially different physiological performance and tolerance ranges to temperature (phenotypes). Previous studies have shown that thermally tolerant corals will include dinoflagellates from clades A to D, which exhibit higher tolerance to thermal stress (Stat *et al.,* 2008). Corals with more tolerant symbionts not only resist the environmental alteration for long time, but have the potentiality to be more resilient and have higher survival rate comparing to other less tolerant symbionts.

In a study conducted in Magnetic Island in Australia (Berkelmans and Oppen, 2006) investigated the adaptation potential of *Acropora millepora,* a common species of hard coral in the Indo-Pacific ocean, using transplantation and experimental manipulation. The authors found that the tolerance level increased with corals changing their dominant symbiont type to D which is known to be resistant to high temperature (Berkelmans and Oppen, 2006). The possibility of coral reefs to survive and tolerate high temperatures due to climate change is very dependent on the corals' ability to adapt and tolerate the extreme temperature conditions. Different symbiotic zooxanthellae species are thermo-sensitive and can withstand the bleaching event, which can potentially lead to the loss of the coral reef. Some corals are able to adapt to high temperature conditions but this tolerance capability is linked to the symbiont type living in their tissue (Backer *et. al*., 2004). This suggests an important ecological significance of corals sheltering different type of zooxanthellae. By transplantation of symbiont resistant type, we can reduce the

impact of global warming through increasing the tolerance level and conserving the reefs particularly the one under threat (Jones *et al.,* 2015).

Another recent study, conducted in the southern Arabian Gulf area, studied the tolerance level of corals in the region (Hume *et al.,* 2015). The authors found that the tolerance level related to specific symbiont type unique to the Arabian Gulf. The symbiotic relationship between the host coral and its zooxanthellae in the Arabian Gulf show an extraordinary temperature tolerance, even throughout the summers when the water temperatures peak up to 36°C. Using molecular markers they found that a newly described species *Symbiodinium thermophilum* is significant for coral survival at the world's hottest sea (Arabian Gulf). (Hume *et al.,* 2015). Many methods have been used to study the photosynthetic activity of the coral such as PAM (pulse amplitude modulated) fluorometry and flow cytometry. A study on the coral bleaching event at Puerto Morelos, Mexico, examined the physiology of dinoflagellates associated with reef-building coral *Orbicella faveolata* during, before, and after the bleaching events (Kemp *et al*. 2014). The authors used PAM fluorometry and molecular genotyping to compare the variability of the dinoflagellate community present during the three stages. The maximum quantum yield of photosystem II (PSII), a proxy measure of photosynthesis, was significantly less during bleaching events compared to other summers when no coral bleaching occurred. The different *Symbiodinium* types were linked to the variable responses to environmental stress. The molecular genotyping of the coral also showed that coral colonies with phylotypes B17 and C7 were more sensitive to the bleaching more than the ones that had phylotypes A3 (Kemp. *et.al,* 2014)

Recent studies have used a less demanding methodology, flow cytometry (FCM) method,

to determine the bleaching responses of the zooxanthellae. Lee *et al*. (2012) studied the response of the zooxanthellae to thermal stress using flow cytometry. They studied the physiological response of the coral and enumeration of zooxanthellae through FCM as a rapid and sensitive method. The differentiation and counting between healthy and unhealthy zooxanthellae was found to be reliable and efficient, as FCM is based on the fluorescence of photosynthetic pigments (red and green fluorescence) and light scatter (forward and side scatter). FCM and microscopy counts of the cells isolates from *Pocillopora damicornis* were compared. The intensities of the photosynthetic pigment fluorescence (chlorophyll and β-carotene) were significantly higher in the fresh unstressed zooxanthellae compare to the cells exposed to high temperatures. Also, the FCM was able to identify and estimate zooxanthellae subpopulations based on light scatter. This features provided an advantage for understanding the involved adaptive and tolerance mechanisms. It was concluded that the approach provided a quick and efficient assessment of the physiological state of corals (Lee *et al.*, 2012). In 2015, a study conducted in USA (Krediet *et al*, 2015) to compare different methodology for Symbiodinium characterization in coral tissue. They have been compare between two methods: counting by using hemocytometer or Guava flowcytometer. The authors found the FCM methods comparing to other available methods is most accurate, precise, and time efficient. In addition, it allows counts of the dinoflagellates cells over extensive range with small sample volume (Krediet *et al*, 2015).

2.1 Research Objectives:

The specific objectives of our study are:

- Assess coral cover and species diversity in an offshore healthy and diverse coral reef
- Assess the physiological response of the different *Symbiodinium* associated with the dominant coral species from an offshore reef in Qatar to temperature stress using laboratory-based experiments
- Characterize *Symbiodinium* subpopulation based on their photosynthetic pigments and relative abundances

CHAPTER 3: METHODS

3.1 Research Strategy

In this study, three reef-building coral species were exposed to different temperature levels. Corals were maintained in aquaria, under controlled artificial conditions, to evaluate their physiological responses to temperature stress. The expected response of coral species to persistent thermal stress is coral bleaching where the symbiotic zooxanthellae is lost from coral tissue. Bleaching can increase the mortality events of the coral reefs (Weis, 2010). Thus, our first aim is to the study the effects of the temperature on the coral-dinoflagellate symbiosis and investigate photosynthetic performance under different temperature conditions by assessing photosynthetic activity of the Symbiodinium zooxanthellae (symbiotic dinoflagellates) throughout the experiment. In addition, our second aim is to characterize the zooxanthellae community and health by measuring their abundance and determining the different photosynthetic pigments present in the corals exposed to the different temperature treatments. To achieve these objectives, thermal stress study was designed and carried out for 35 days after a two-week period of acclimatization at ambient temperature with considering only one stress (temperature) while keeping other variables constant (hydrological and light exposure). The experiment consisted in three sets of temperature ranges: 25(± 1)^{o}C as control , 30(±1)^{o}C, and 35 (± 1)^{o}C , with one replication of each set. Temperature was gradually increased by one degree per day on week days for the two temperature treatments, using aquarium heaters with thermostat. Three coral species were selected for the experiment with very different range of physiological response to environmental stress. *Porites* species is known to be tolerant, *Acropora* species is known to be sensitive and *Dipsastraea* species *i*s known to

have intermediate tolerance sensitivity (Howells *et al.*, 2011 and Furby *et al.*, 2013). To maximize replication in the experiment, the experimental units (aquaria) were duplicated, and in every experimental units, each fragment of the three coral species was replicated (n=3). Water quality and chemistry were monitored and maintained as constant as possible throughout the duration of the experiment through periodic water renewal. Diving-PAM (Pulse-amplitude modulation) was used to assess the photosynthetic activity of the zooxanthellae. At the end of the experiment, the zooxanthellae community was quantified and characterized using flow cytometry and enumeration under light microscopy.

In order to assess the significance of this work to existing healthy coral reefs offshore of Qatar, a characterization of the source coral reef was conducted to understand original coral health and coral cover in-situ.

3.2 Coral Sampling

Coral samples were collected from an offshore reef (North of Qatar), Um Al-Arshan (Figure 3). The coral collection was performed by expert coral specialist divers.

Figure 3: The collection site of coral samples (Um Al-Arshan)

Three coral species were selected for the experiment with known different range of physiological response to environmental stress: *Porites lutea*, *Acropora downingi* and *Dipsastraea pallida* (Howells *et al.*, 2011 and Furby *et al.*, 2013). Small fragments (7-20 cm) of the coral were collected from the mother colony using a hammer and chisel. The coral samples were transported from the field to the lab in wide container with water from the sampling site.

3.3 Regional setting of the studied species

Coral assemblages were surveyed at the offshore reef Umm Al-Arshan in Qatar over the course of this study. 33 photoquadrats were taken on SCUBA by professional divers (Figure 4). At each sampling site (colony), three replicate 30 m transect tapes were laid

out parallel along the reef bottom and spaced approximately 5 m apart. High-resolution photographs were taken using a frame-mounted underwater camera encompassing a quadrat area of 0.25 m^2, with images taken at 3 m intervals along each transect, for a total of 33 high-resolution benthic images at each site. Each photoquadrat was analyzed using 50 point counts to demonstrate_the abundance of live coral (hard coral cover), the diversity of the three studied coral species (species richness), and other major benthic habitat categories (e.g. algae, sponges, substrate types). Images analysis was done using CoralNet, http://coralnet.ucsd.edu, an online resource and repository for benthic images analysis. Fifty random points were analyzed per image in order to estimate cover and identity of corals and other benthos in each image. This quantitative approach has been successfully used to survey coral reefs at several locations in the region, including numerous reefs in Qatar, UAE, Bahrain, Kuwait and Oman (Burt *et al.*, 2008; 2011; 2013). This task allowed the identification of key coral species and their respective cover percentage among all other benthic communities.

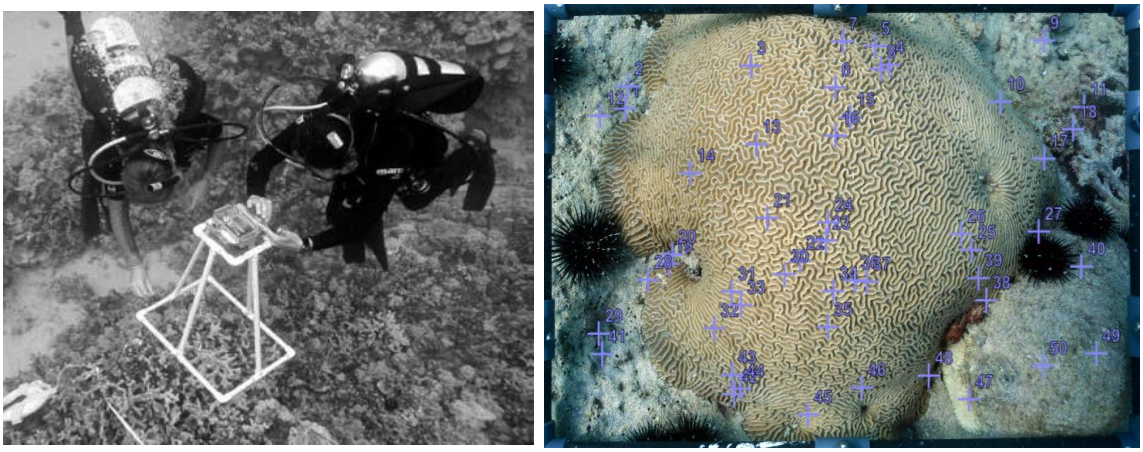


Figure 4: Underwater photographs acquisition and analysis of the photoquadrats using CoralNet

3.4 Coral acclimatization and maintenance

All aquarium experiments were conducted at ExxonMobil Research Center (EMRQ) in Qatar. The corals were carried in box filled with seawater from the collection site and delivered to the laboratory ~4h after sampling then it distributed among the laboratory aquariums. The initial seawater parameters were set to reproduce as much as possible the original conditions at the sampling site: salinity: 40 psu and water temperature 24 °C.

3.4.1 Acclimatization, water parameters, and light setting

All aquariums were filled out with artificially mixed seawater using commercially available aquarium sea salt and demineralized water. Water chemistry and salinity were maintained and monitored to be close to natural seawater of the collection site. Water chemistry parameters (nitrate, nitrite, ammonium, phosphate, magnesium, and calcium) were measured three times per a week using a JBL© water test kit, Germany. PH was measured using a HACH pH meter, and pH buffer was added to keep the range of the pH between 8-8.3. Salinity was measured using a handheld refractometer. Temperature was

measured manually using the temperature sensor on the HACH pH meter or a digi-thermometer. Each aquarium was provided with a separate light system using LED aquarium lights with two light spectra (white, and royal blue) (Table 1). All aquariums were set up with separate filter units and skimmers.

Table 1: Aquarium Light Setting

Time	**Percentage of white light**	**Percentage of Royal blue light**
5:30 AM	20%	50%
8:00 AM	20%	40%
4:00 PM	60%	100%
5:30 PM	60%	100%
6:00 PM	0%	0%
11:30 PM	0%	1%

3.4.2 Maintenance and feeding

Each aquarium was ran on a closed-system with separate filtration units and skimmers to remove debris, circulate water and mimic flow current in order to maintain appropriate water quality (Figure 3). All filters were cleaned once a week to avoid any blockage of the filtration units, and all skimmers were monitored and cleaned every two days per week by adjusting them and removing the dirty water.

Figure 5: Coral acclimatization and maintenance in laboratory aquaria

Water change was done once to twice per week depending on the water chemistry. The percentage of the water changes was 20% of the water or higher depending on the water quality. Aquariums which were assigned the higher temperatures were set up with aquarium heaters. Control aquarium were left at ambient temperature 25°C (lab air temperature = ~22°C). Corals were feed once a week using Coralific Delite.

3.5 Temperature Experiment

Three replicate 100 L aquaria were used for each experimental temperature (25, 30 and 35°C). To avoid pseudo-replication, adequate replications of the experimental units (aquaria) were considered while using several replicate coral species per experimental unit to increase the precision of our estimates (Table 2). Figure 5 illustrates the replication of each species in each tank. Temperature was increased by 1°C per day by placing heaters with thermostat into the aquaria. After a two-week period of acclimatization at ambient temperature, the temperature experiment then started and ran for 35 days at constant salinity (40±2 psu) and light intensity (6900 Lux) at ExxonMobil

Research Qatar. PAM (Pulse Amplitude Modulated fluorometry) measurements were taken twice a week until the end of the experiment. Every time, 3 replicates of PAM measurements were taken for each colony.

Table 2 : *The Setup of the Experiment*

25°C **3x of 3 species**	**35°C** **3x of 3 species**
30°C **3x of 3 species**	**30°C** **3x of 3 species**
35°C **3x of 3 species**	**25°C** **3x of 3 species**

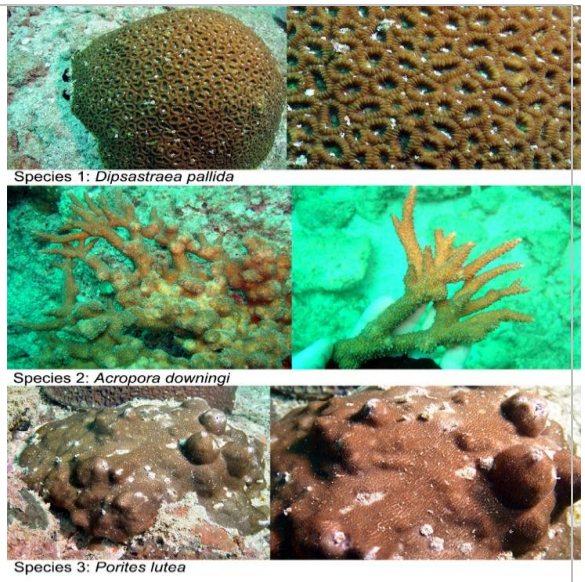


Figure 6: illustration of the three species added in each tank (with replication n=3)

3.6 Assessment of photosynthetic activity using a Diving PAM fluorometer

Chlorophyll *a* fluorescence of the coral zooxanthellae was measured using a pulse-amplitude modulated fluorometer (PAM; Walz, Effeltrich©, Germany). This instrument was used in the lab to measure maximal quantum yield of photosystem II after dark adaption. The saturation pulse prompts maximal fluorescence yield, Fm, and maximal variable fluorescence, Fv=(Fm-Fo). Maximum Quantum Yield Fv/Fm, is a reliable measure of the potential quantum yield of PS II. PAM measurements were taken twice a

week over 35 days for the surviving colonies or until the colonies died (Figure 7). PAM measurements were taken early afternoon in a dark environment, with aquarium and laboratory lights switched off for an hour before every run of PAM measurements. It has been shown that an hour of darkness is sufficient to reset photosystem (Salih. *et.al*, 2006).

Figure 7: PAM measurement equipment and technique

3.6.1 Rapid light curve:

A series of rapid light curve (RLC) measurements were performed on some colony from the three species under different treatment. Rapid light curves (RLC) measure effective quantum yields over a range of increasing light intensities. Therefore, they provide additional information about the efficiency and capacity of photosynthesis by illustrating the acclimation of the photosynthetic apparatus (Belche et al. 2007).

3.7 Characterization of the zooxanthellae

3.7.1 Extraction of the zooxanthellae from the coral tissue:

Zooxanthellae cells were extracted from the coral colonies for subsequent flow cytometry

and haemocytometry analyses. A small fragment was sampled from each coral in the aquarium using a hammer and chisel for the extraction of the zooxanthellae. Samples brought to Qatar University (QU) on the afternoon before the extraction day and were kept in 0.22 µm filtered seawater overnight in small containers with an air stone to keep the water in movement and oxygenated (Hagedorn . *et.al*, 2010) (Figure 8).

Figure 8: Left: subsamples kept in small containers overnight until zooxanthellae extraction. Right: filtering seawater

All zooxanthellae extractions were conducted within 4 hours prior to the flow cytometry analysis to ensure that the zooxanthellae remained alive for the analysis. For each subsample, coral tissue was blasted from the coral skeleton using a WaterPik and 0.22 µm filtered seawater. The removed tissue was then poured into 50ml falcon tubes to be centrifuged. Each sample was run three times in the centrifuge at 6000 rpm for 15 mins. After every run, the supernatant was discarded and the pellet was re-diluted in filtered

seawater. After the last run, all samples were thoroughly vortexed to ensure homogeneity (Hagedorn. *et.al,* 2010).

3.7.2 Characterization using flow cytometry

All samples were analysed using the BD LSRFortessa flow cytometer. One milliliter aliquots of sample was used for each run; subsamples were taken from the same sample to be processed for microscopy count. Each sample was contained thousands of cparticles suspended in water consisting of zooxanthellae cells and other microorganisms. In flow cytometry, we gated or identified the zooxanthellae cells based on the sizes and shapes (50-100 μm). Forward scatter (FSC) represents the cell size and detected by photodiode detector. Side scatter (SSC) represents different shapes of the cell and collected via 488±6nm band-pass filter. FITC-A (Fluorescein isothiocyanate) fluorescence signals from chlorophyll *a* were collected via 695 ±40-nm band-pass filter (Lee *et al*., 2012). PerCP-A (Peridinin-Chlorophyll-protein) fluorescence signals from β-carotene were collected via 529 ±28-nm band-pass filter. Data acquisition and analysis were performed using the BD FACSDiva software. Morphologically different *Symbiodinium* can be differentiated using forward scatter and side scatter. Their physiological state can also be characterized based on their pigments' auto-fluorescence.

3.7.3 Enumeration using haemocytometry

All extracted samples of zooxanthellae cells were examined under the microscope and enumerated. The zooxanthellae were counted using a Neubauer Haemocytometer under light microscope. Healthy and unhealthy zooxanthellae were quantified under 40X magnification objectives using the light microscope BX43 (Olympus©). The cell counts were standardized to $1cm^2$, after calculations of the exact surface area from which coral

tissue had been extracted.

3.8 Data Analysis

Data were analyzed using analysis of variance (ANOVA) to assess the statistical difference between the coral species responses to temperature stress (treatments). Variations in the Maximum Quantum Yield (YII) for all samples in different treatments were determined in Excel.

CHAPTER 4: RESULTS

4.1 Regional setting of the studied species:

This approach has been used to study coral reefs coverage at Um Al-Arshan. The hard coral cover in that area was 18%, 36% was covered by algae, and 44% was covered by sand and rubble (Figure 9). The percentage of three studied species (*Porites lutea*, *Dipsastraea pallida*, and *Acropora downingi*) in the study are was 15%, 12 %, and 4% respectively (Figure 10).

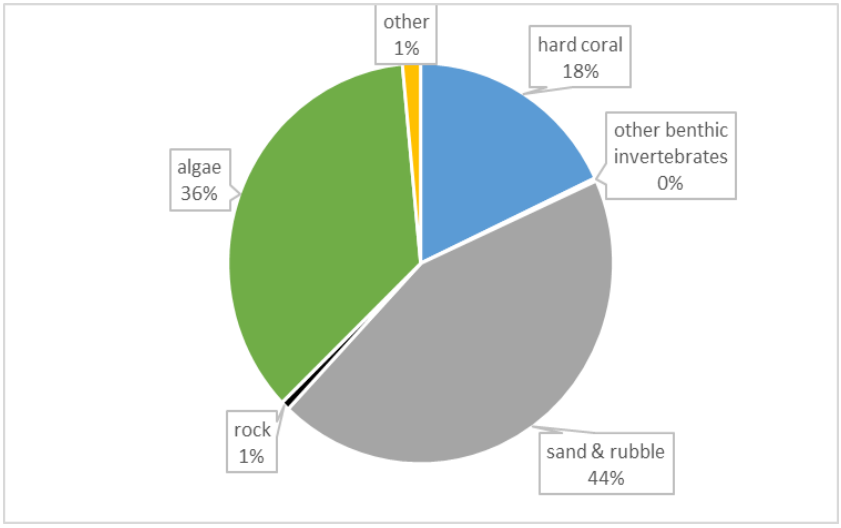


Figure 9: benthic community cover at Um Al-Arshan reef

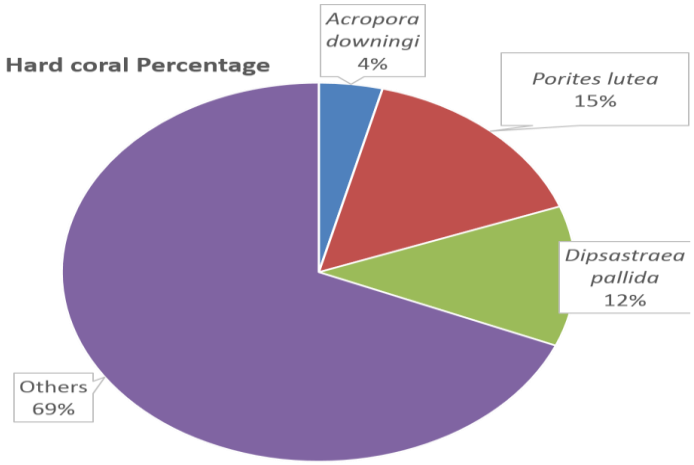


Figure 10: Hard coral percentage including the three studied species at the study area.

4.2 Water Chemistry

Water parameters were monitored throughout the duration of the experiment. The following parameters were measured at least once a week: salinity, pH, temperature, nitrate, nitrite, ammonium, phosphate, magnesium, and calcium. After a two-week period of acclimatization at ambient temperature, temperature was gradually increased by one degree per day on week days (Sunday to Thursday) for the two temperature treatments, using aquarium heaters with thermostat. Each coral colony was visually monitored every day and showed no sign of stress from the rate of temperature increase. Salinity was kept at 42±2 psu, as per the salinity range found in reef ecosystems in Qatar and to avoid any stress other than thermal stress. pH was monitored and kept within 8.0-8.3 as much as possible, using reef buffer to increase pH when necessary. Nitrates (NO_3) were maintained under 0.5 mg/l almost throughout the entire experiment; however, an increase was noted in some aquarium tanks towards the end of the experiment, following an

increase in ammonium and nitrites linked with the bleaching and subsequent death of *Acropora* colonies in the hotter treatments. Nitrites (NO_2) were generally within acceptable range (0.025-0.05 mg/l), but they reached 0.4 mg/l towards the end of the experiment in the hottest tanks following the bleaching and rapid subsequent death of *Acropora* colonies. Ammonium (NH_4) was within the acceptable range during the experiment (<0.05 mg/l), although slightly higher in the hottest tanks towards the end of the experiment following the death of *Acropora* colonies. To reduce the levels of nitrates, nitrites, and ammonium, up to 50% water renewals were conducted in each aquarium in addition to the weekly 20% water changes. All monitoring results of water quality parameters and figures are reported in Appendix I.

4.3 PAM results

4.3.1 Accuracy of PAM measurements replicates

Each PAM measurement was conducted on a surface of approximately $1cm^2$. In order to verify that three replicate maximum quantum yield measurements are representative of the entire colony surface, a power analysis was done comparing the average and standard deviation for three replicates and for ten replicates (Figure 11). The analysis showed no significant difference between the data from three replicates and the data from ten replicates (Welch's t-test, p-value > 0.05, Figure 10).

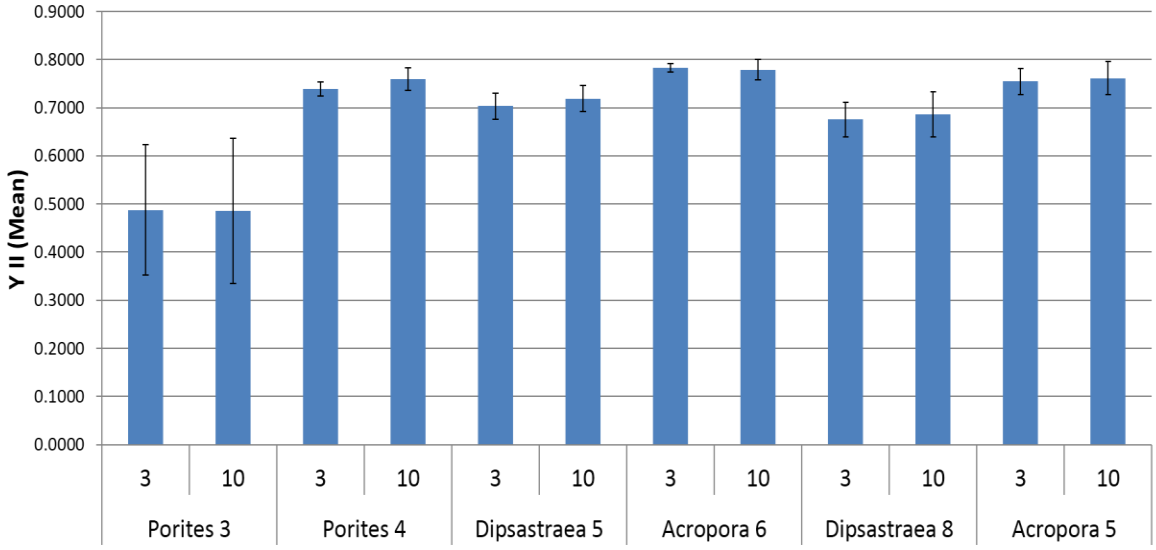


Figure11: Comparison on means and standard deviations between three replicate measurements, and ten replicate measurements of the three coral species: *Porites* (P), *Dipsastraea* (D) and *Acropora* (A). The numbers represent the different colonies that were selected.

4.3.2 Maximum Quantum Yield (YII)

The maximum quantum yield Y(II) was monitored throughout the duration of the experiment to detect any change in the maximum photochemical efficiency of photosystem II (PSII) (Figure 12).

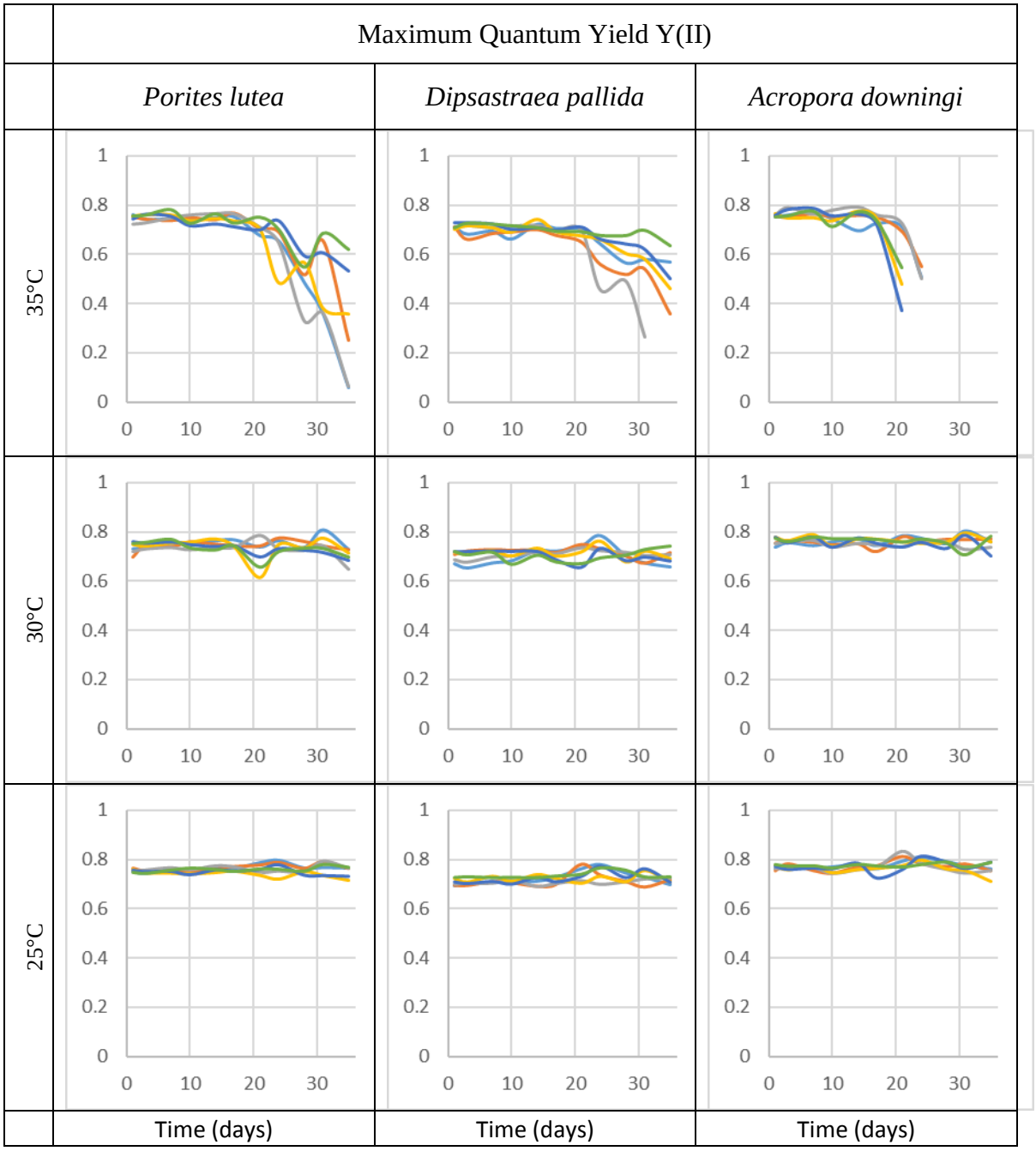


Figure 12: Maximum quantum yield of individual colonies in each treatment (rows) and for each species (columns). Different colored lines represent the replication of each colony species n=3 in the two duplicated experiment units n=2.

Response of each studied species to the temperature treatments

Porites lutea maintained its maximum quantum yield throughout the duration of the experiment in the 30°C treatment and in the 25°C control; however, , Y(II) significantly decreasing between the three treatment on 17 days ($P < 0.05$) (Figure 13). *Dipsastraea pallida* also maintained its maximum quantum yield throughout the duration of the experiment in the 30°C treatment and in the 25°C control; and Y(II) also started significantly decreasing on 17 days ($P < 0.05$) (Figure 14). Finally, *Acropora downingi* maintained its maximum quantum yield throughout the duration of the experiment in the 30°C treatment and in the 25°C control; however, Y(II) started significantly decreasing between the three treatment on 17 days ($P < 0.05$) (Figure 15). In the latter treatment, bleaching was also apparent after 21 days, shortly before the colonies died after 24 days.

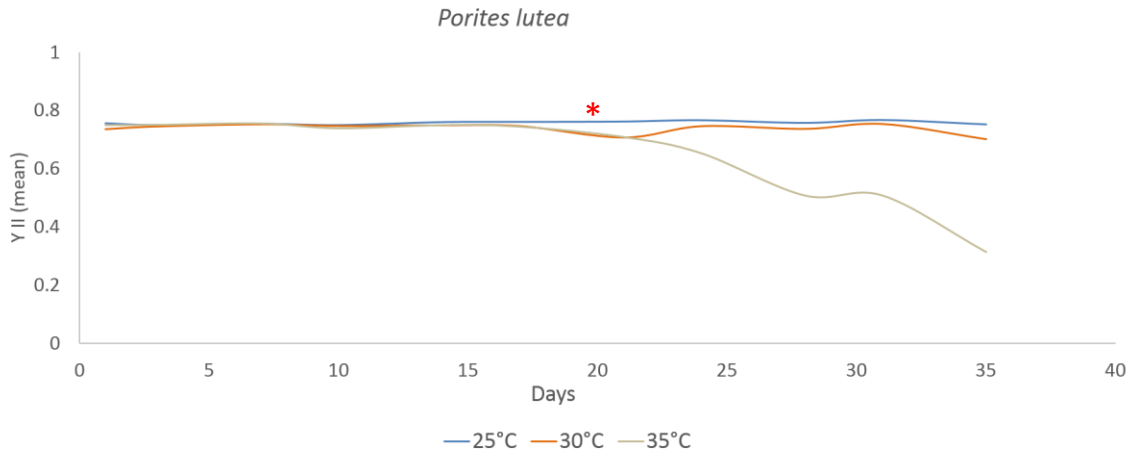


Figure 13: Maximum quantum yield of *Porites lutea* at 35C, 30°C, and 25°C (control) throughout the experiment

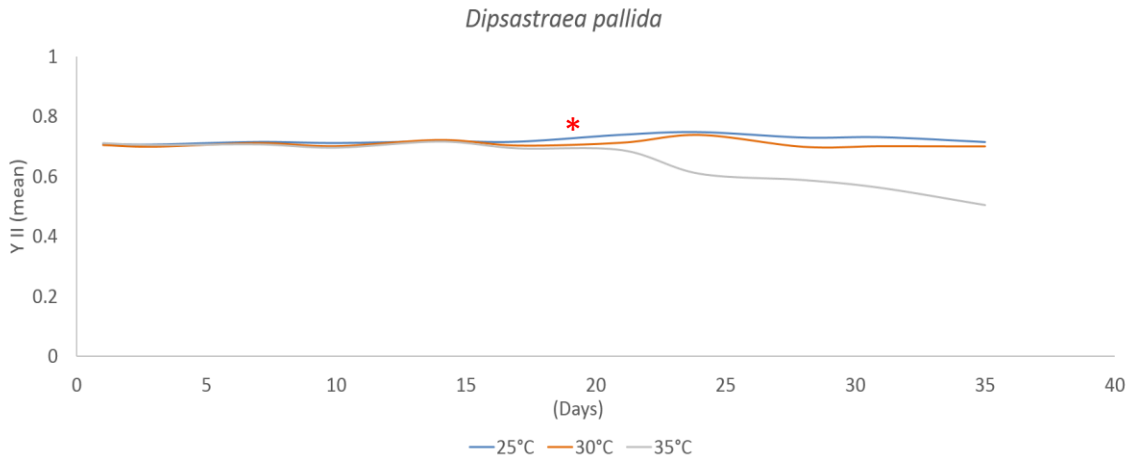


Figure 14: Maximum quantum yield of *Dipsastraea pallida* at 35°C, 30°C, and 25°C (control) throughout the experiment

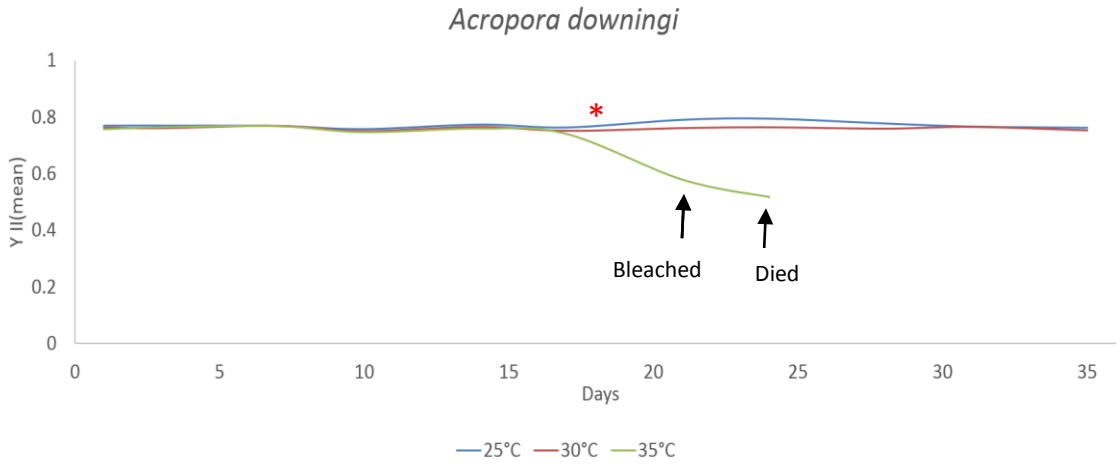


Figure 15: Maximum quantum yield of *Acropora downingi* at 35°C, 30°C, and 25°C (control) throughout the experiment

Differences and similarities among the three studied species

None of the three species appeared to be affected by the 30°C temperature treatment. However, they did show a different response to increased heat and different temperature tolerance duration (Figure 16). *Acropora downingi*, has a threshold level once they were exposed to high temperature (35°C), they bleached after 21 days and died only 3 days after they bleached (24 days). In contrast, Maximum quantum yield of *Porites lutea* was strongly reduced with higher temperature (35°C), but the coral colonies remained alive and not bleached throughout the experiment, suggesting high tolerance to high temperatures. *Dipsastraea pallida* showed an intermediate response compared to the other two species, but it was still affected at 35°C temperatures.

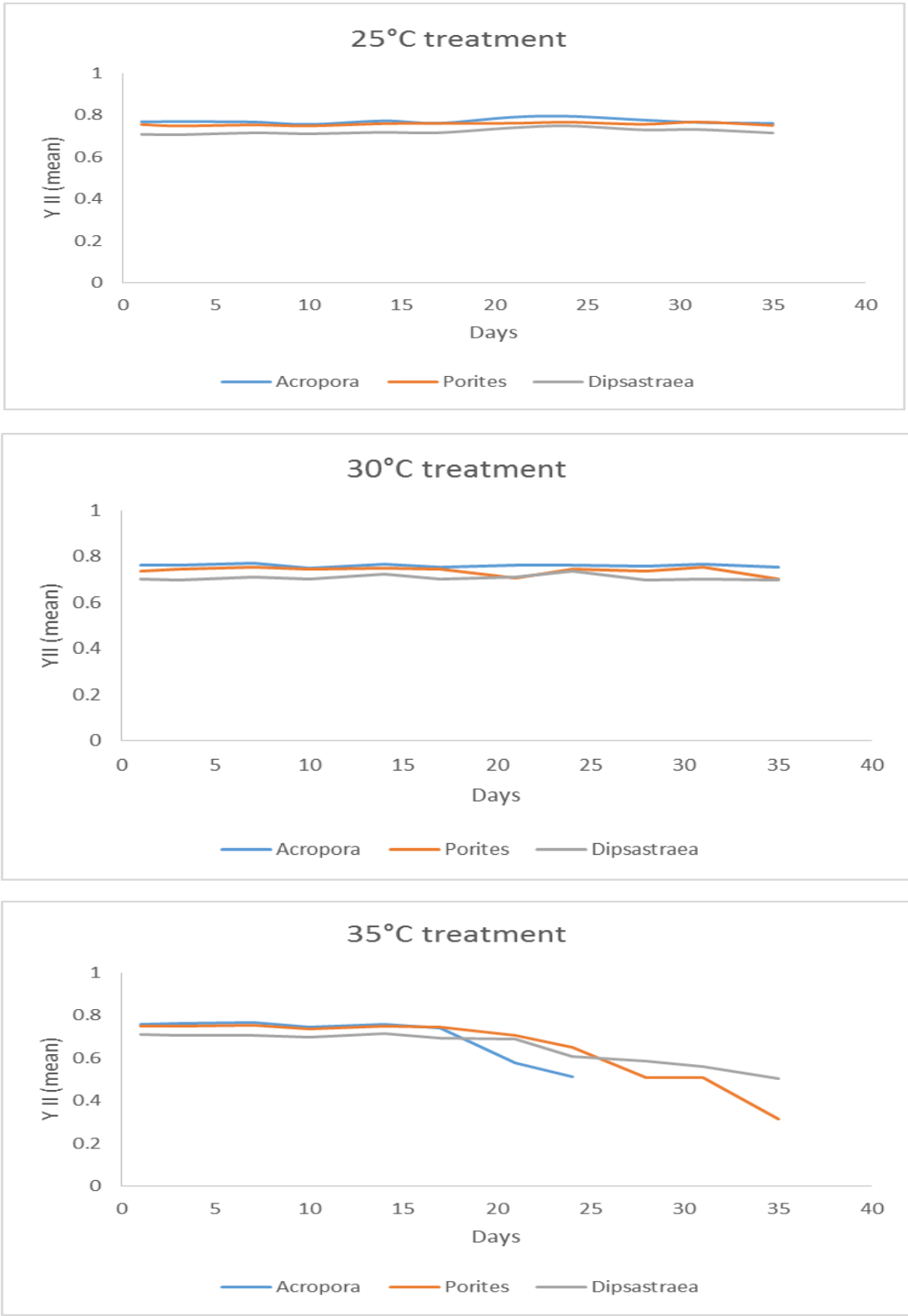


Figure 16: Species-specific tolerance and susceptibility

4.3.3 Rapid light curves

The rapid light curves were conducted close to the end of the experiment, on day 38. Unfortunately, by that day, only *Dipsastraea pallida* had survived the heat stress. The light curves in *D. pallida* behaved in a similar way in all treatments, but showed much lower effective quantum yield in the 35°C treatment throughout the light curves (Figure 17). The light curve of *Porites lutea and Acropora downingi* showed similar performance in 25°C and 30°C treatment; however no *Porites* or *Acropora* colonies were survived in 35°C treatment (Figure 18 and 19).

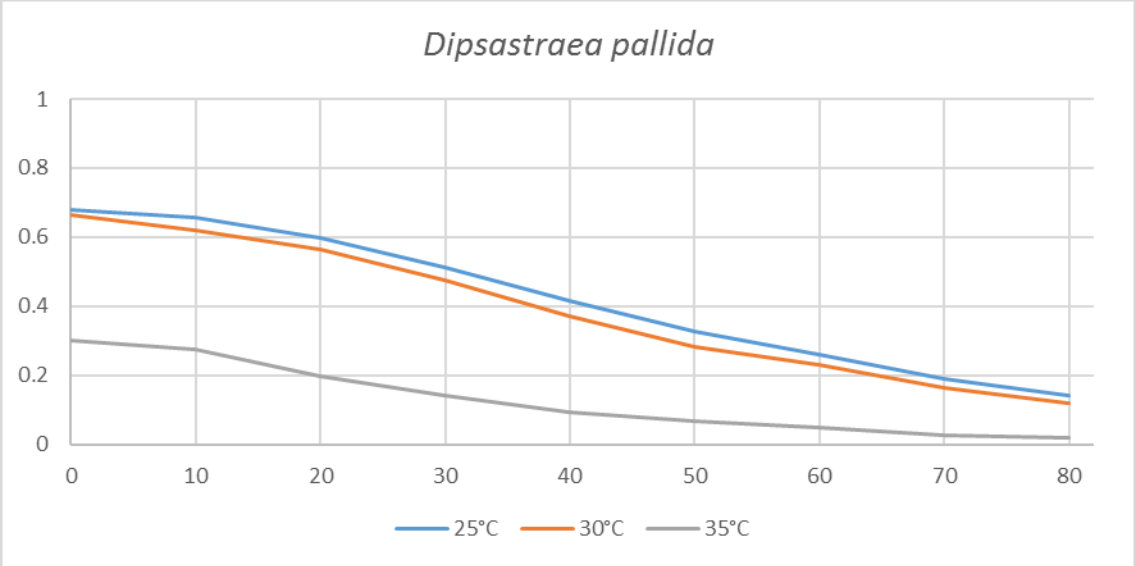


Figure 17: Rapid light curves in *Dipsastraea pallida* under all three treatments

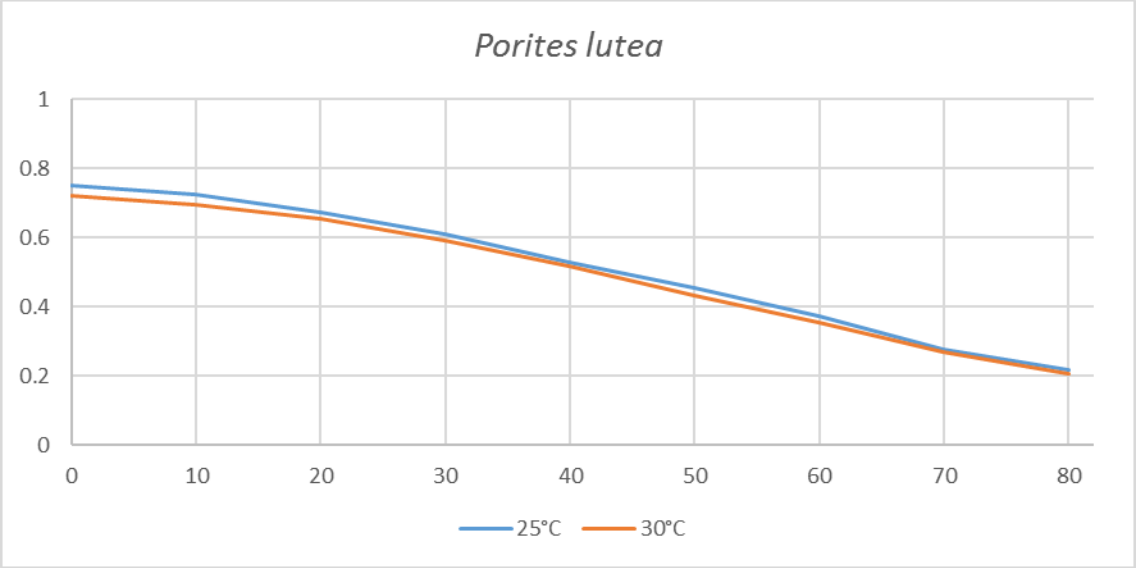


Figure 18: Rapid light curves in *Porites lutea* under the two treatments (25 and 30°C)

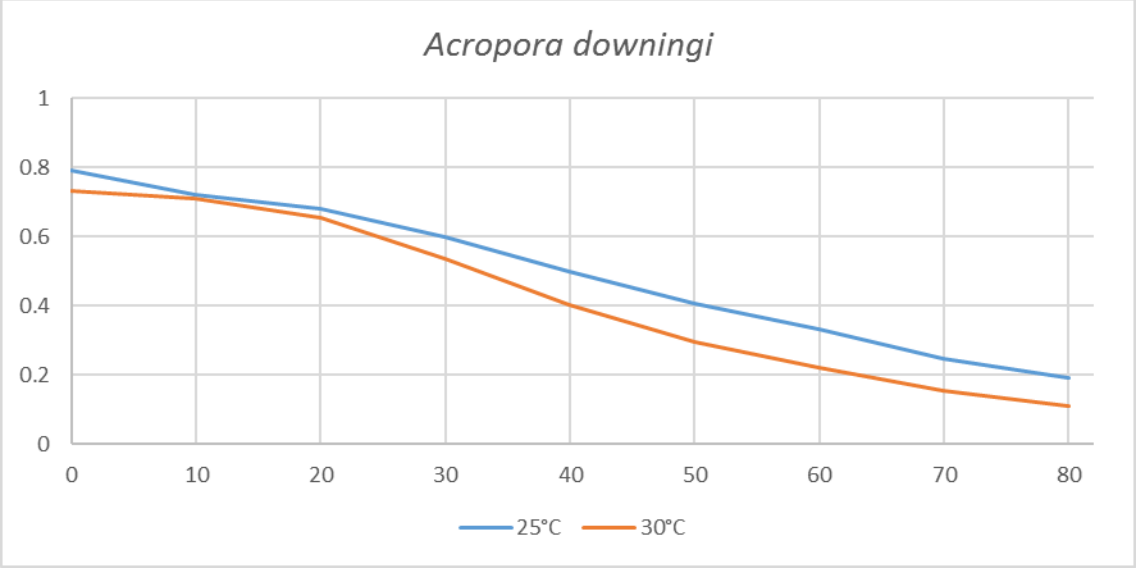


Figure 19: Rapid light curves in *Acropora downingi* under the two treatments (25 and 30°C)

4.4 Characterization of the zooxanthellae through flow cytometry

Flow cytometry was used to separate subpopulations and to differentiate between healthy and unhealthy zooxanthellae cells using photosynthetic fluorescence signals (Figure 21).

No data showed for *Acropora downinigi* in the 35°C, as they have been lost due to high temperature before the flow cytometry experiment started. *Porites lutea* at 25°C and 30°C showed no big difference between the number of healthy and unhealthy cells. However, it presented big increase of unhealthy cells between *25*°C and 35°C from 34.47% to 99.85% respectively. *Dipsastraea pallida* showed decreasing of the healthy cells counts throughout the three treatments (25°C, 30°C and 35°C). *Acropora downinigi* did not show apparent differences between healthy and unhealthy cell in both treatment (25°C and 30°C) (Figure 21). Figure 20 illustrate the results obtained from flow cytometry analysis, where the zooxanthellae were identified based on the size (50-100 □m) and differentiation between the healthy and unhealthy cells was based on the Chlorophyll-*a* and β-carotene fluorescence. Unfortunately, forward (FSC) and sider scatter (SSC) were not able to separate any zooxanthellae subgroups, as what have been found in (Lee *et al*., 2002) suggesting only one type of zooxanthellae was enfolded within the coral tissue of the three species. All Flow cytometry analysis on the three species and under the three experimental treatments are provided in Appendix II

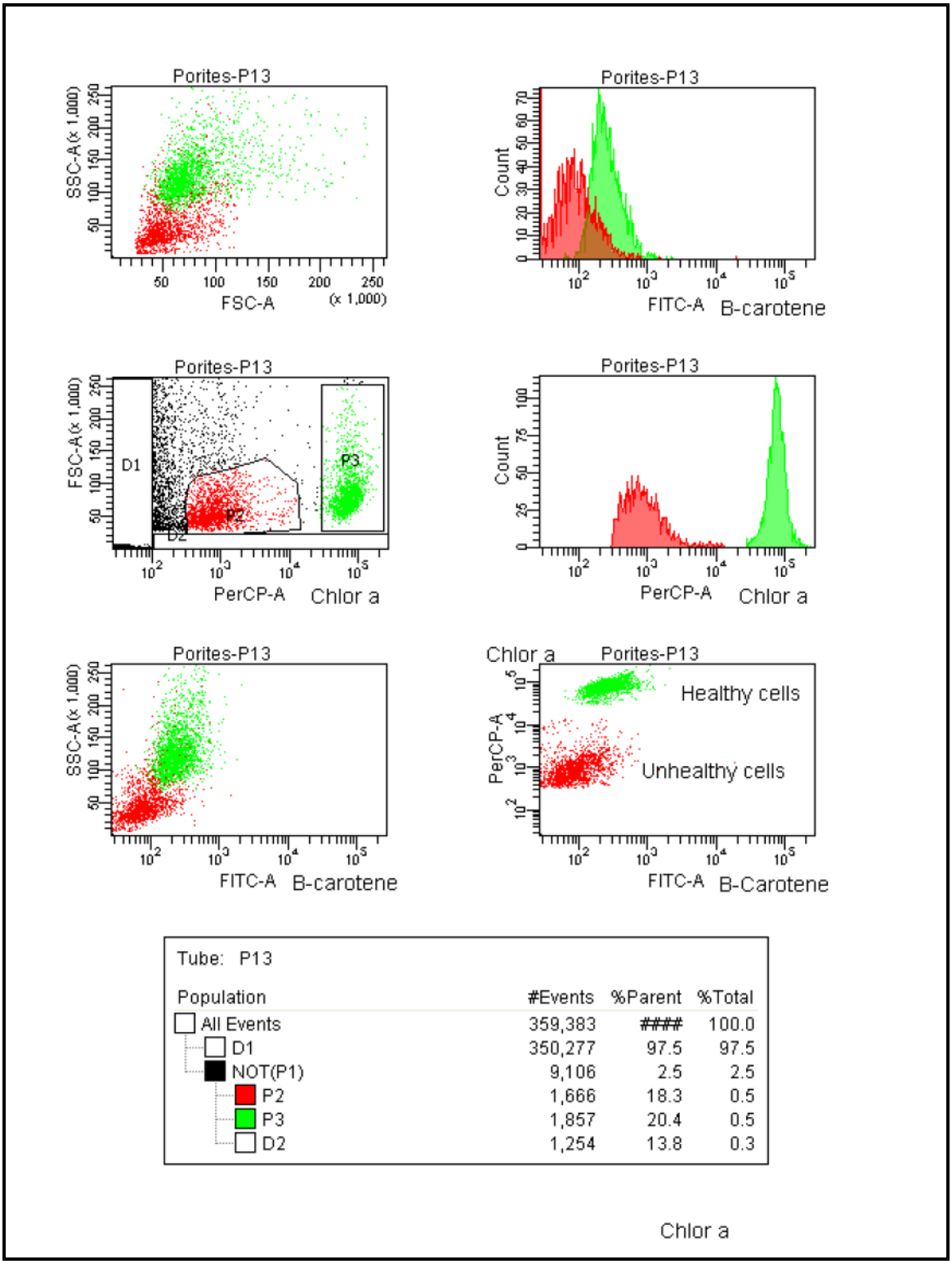


Figure 20: Flow cytometry results of one sample *Porites lutea* at 30°C

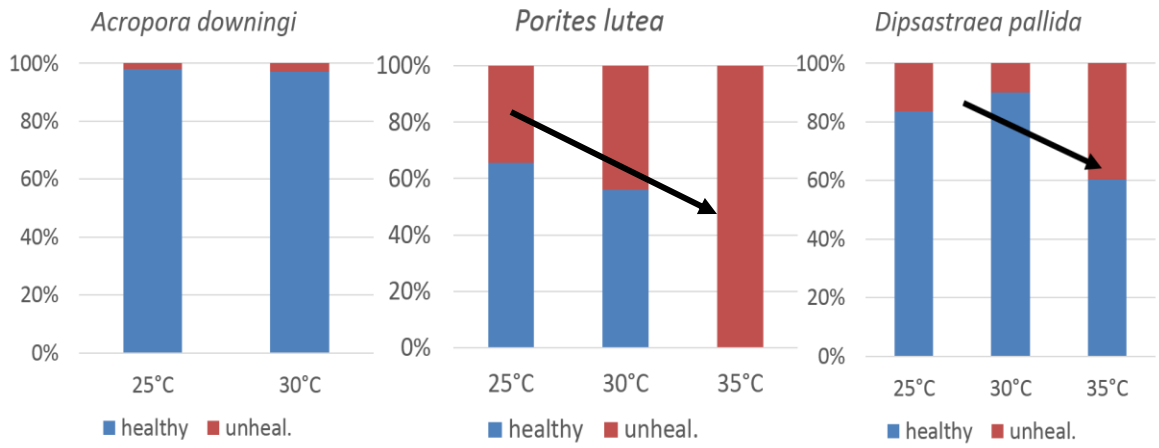


Figure 21: cell proportion per species (*A. downingi*, *P. lutea*, and *D. pallida*) and per status (healthy and unhealthy) from the flow cytometry analysis

4.5 Enumeration of the zooxanthellae using haemocytometry

In addition to flow cytometry, enumeration of the zooxanthellae was done by haemocytometry under light microscope to assess the effectiveness of the use of flow-cytometry in differentiating between zooxanthellae cells status (healthy vs unhealthy). *Porites lutea* showed increased counts of unhealthy cells between the three experiment treatment (25°C, 30°C and 35°C). *Acropora downinigi* showed slight decreased counts of the unhealthy cells at 30°C compared to 25°C treatment. In contrast, largest cell count of the unhealthy cells of *Dipsastraea pallida* was in the 35°C treatment comparing to the other two treatments. (Figure 22).

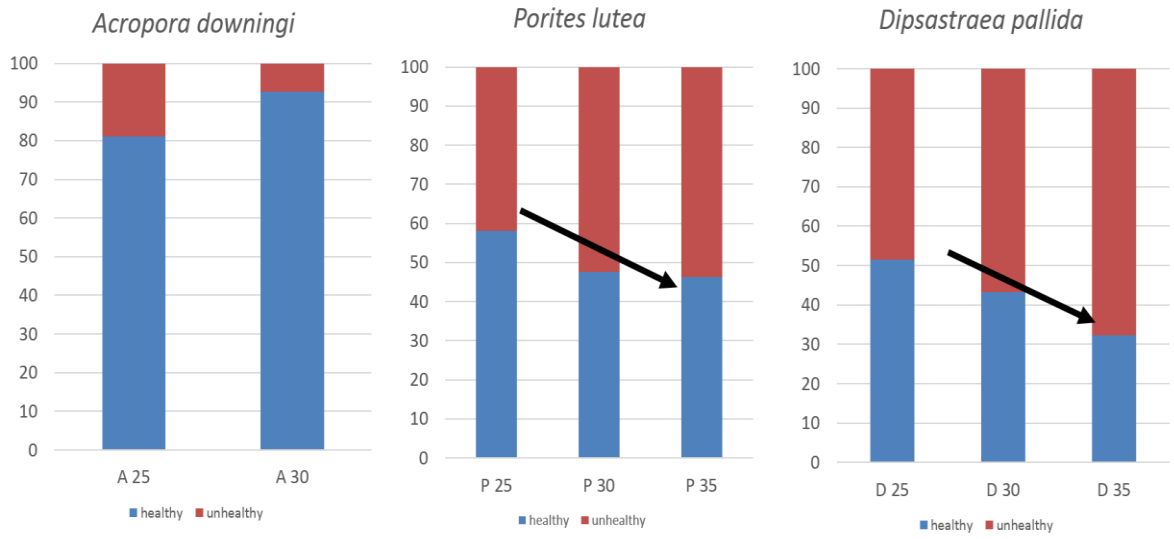


Figure 22: Cell proportion (*A. downingi*, *P. lutea*, and *D. pallida*) and per subpopulation (healthy and unhealthy) from the haemocytometry counting

CHAPTER 5: DISCUSSION

Studying coral reefs is very important especially in an area such as the Arabian Gulf, where they are subjected to extreme temperatures and experiencing mass mortalities during the last decades (Sheppard, 2012). One of the greatest stressor corals in the Arabian Gulf are facing, is increasing seawater temperature (i.e. ocean warming) as corals in the Gulf are very close to their maximum temperature thresholds (Shuail *et al*. 2016). High temperature can cause photosynthetic zooxanthellae to be expelled from the coral tissue leading to coral bleaching followed in some cases by mortality (Berkelmans and Oppen, 2006). 80% of the corals' nutritive intake depends on the photosynthetic product from the symbiotic zooxanthellae (NOAA, 2007). This study showed that the photosynthetic activity of the zooxanthellae declines after exposure to an extended period of thermal stress, defined here as a temperature in the 35-36°C range, without any visual signs of bleaching. Our study reproduced natural thermal stress that occurs each summer to coral reefs in Qatar (Figure 30; data retrieved from MODIS-Aqua (Day) Giovanni online data system, developed and maintained by NASA - GES DISC). Many inshore reefs, where shallow waters heat faster than in the deeper waters surrounding offshore reefs, have suffered from coral bleaching and subsequent mortality (Burt *et al*. 2016; Bouwmeester and Ben-Hamadou, unpublished data). The results of this study showed that the three species included in this study (*Porites lutea*, *Dipsastraea pallida and Acropora downingi*) were affected by the high temperature (35ºC) treatment.

Figure 23: averaged map of sea surface temperature in July to August in the Arabian Peninsula (map retrieved from MODIS-Aqua (Day) Giovanni online data system, developed and maintained by NASA - GES DISC)

Results revealed that high temperatures negatively affected the zooxanthellae photosynthetic activity on the three species. Also, *Acropora downingi* lost photosynthetic activity under 30ºC treatment. Our findings are similar to those from previous studies (e.g., Kemp *et al.*, 2014), in which the maximum quantum yield of photosystem II (a proxy for photosynthetic activity) from symbiotic dinoflagellates was significantly reduced during bleaching events comparing to values from other summer when no coral bleaching occurred. It is known that different coral species respond differently to thermal stress (Howells *et al*., 2016). Therefore, we selected for our experiment three species with potentially very different physiological responses to environmental stressors, in order to compare the differential species-specific responses to temperature stress. To ensure the survival of coral reef ecosystems in the Arabian Gulf, an appropriate species diversity needs to be fostered for potential protection a higher recovery potential by local environmental managers. A minimum level of biodiversity is required to ensure that

ecosystem services are maintained, even if a species is lost. For example, *Acropora* has become very rare in the Gulf due to their sensitivity to thermal stress in an environment that experiences thermal stress nearly every summer (Riegl, 2002).

The bleaching event that occurred in 2002 in the Arabian Gulf, when the sea temperature reached 37°C, witnessed a lower bleaching rate of the species *Acropora downingi*, *Acropora clathrata* and *Porites harrisoni* comparing to the bleaching rate of other species (Riegl, 2002).

Table 3: Genera-specific Coral Stress Resulting from High Temperatures 1995-2010 (reproduced from Riegl, (2012)).

Genera-specific coral stress resulting from high temperatures 1995–210.

Event	Observations during and after event
1996	*Acropora* spp.: Extensive bleaching, mortality 75–95% (George and John 1999) to total mortality, reducing total species from 34 to 27 (Riegl, 1999, 2002a,b)
	Columnar *Porites* (*harrisoni*): Moderate bleaching and mortality (Riegl, 2002a,b)
	Massive and encrusting *Porites, Cyphastrea, Favia, Platygyra* and *Siderastrea*: No visible stress ((Riegl, 2002a,b)
1998	*Acropora* spp.: Little to no mass bleaching or mortality (Riegl, 2002a,b, 2003)
	Columnar *Porites:* Moderate bleaching and mortality (Riegl, 2002a,b)
	Massive and encrusting *Porites, Cyphastrea, Favia, Platygyra* and *Siderastrea*: 80–95% bleaching (George and John 1999), No visible stress or mortality((Riegl, 2002a,b)
2002	*Acropora* spp: Moderate to no bleaching
	Columnar *Porites*: Moderate bleaching and mortality, exceeded observed for *Acropora* (Riegl, 2003)
	Massive and encrusting *Porites, Cyphastrea, Favia, Platygyra*: Bleached but no mortality (Riegl, 2003)
2010	*Acropora* spp.: Mean% colonies bleached ca. 70% mortality 50–90% from disease (Riegl et al., 2011)
	Porites spp.: Mean% colonies bleached ca. 60%, <10% mortality
	Massive and encrusting *Porites, Cyphastrea, Favia, Favites, Platygyra*: Mean% colonies bleached ranged 15–80% with <10% mortality, *Coscinaraea,Turbinaria, Anomastraea*: 50–100% bleached

These results suggest that the species which were originally sensitive to thermal stress had adapted after the exposure to temperature stress from the first and second bleaching events. Our results have shown that the temperature tolerance of *Porites lutea* was much higher than *Acropora downingi*. Indeed, *Porites lutea* was alive until the last day of the experiment (35 days) although they photosynthetic activity had decreased with the continuous exposure to high temperature (up to 35°C). *Acropora downingi* was very

sensitive to high temperatures, once the temperature reached 35°C at 24 days, they bleached and died two days later. In contrast, *Dipsastraea pallida* showed overall lower photosynthetic activity from day 1 when the temperature was 25 ºC, but did not even bleached by the end of the experiment. Corals in the Arabian Gulf are more adapted to extreme temperature compared to corals from other regions (Howells et al., 2016). Indeed, we found that all three studied species were unaffected by the exposure to 30°C, and that they could survive for an amount of time at 36°C that is species specific (e.g., *Acropora downingi* died after 24 days at 35°C, while *Porites lutea* and *Dipsastraea pallida* survived throughout the entire experiment). Outside the Arabian Gulf, corals are known to start bleaching at much lower temperatures for the same duration of exposure (Shuail *et al*., 2016).

Different approaches have been used to characterize the zooxanthellae cells from different temperature exposures and to discriminate between healthy and unhealthy *Symbiodinium* cells. In our experiment, we used two methods for the characterization of the zooxanthellae: flow cytometry and microscopy. *Porites lutea* showed an increase in unhealthy cells with the increase of temperature from 25ºC to 35ºC. These results were in agreement with the PAM fluorometry results where the maximum quantum yield was substantially less at 35ºC than at 25ºC. No differences between healthy and unhealthy zooxanthellae were found for the three species between 25ºC and 30ºC, probably due to the limited number of samples analyzed by the time of the writing of this thesis.

In addition to flow cytometry, the enumeration of healthy and unhealthy zooxanthellae was performed using haemocytometry under microscope. *Porites lutea* showed an increase in unhealthy cells from the 25°C treatment to the 35°C treatment. Comparing the

different methods for studying the temperature effects on the coral, flow cytometry were found more accurate and efficient as it takes into account the fluorescence of photosynthetic pigments to differentiate between healthy and unhealthy cells, while PAM fluorometry gives the overall photosynthetic activity of the coral tissue (Table 4). Flow cytometry has been shown as effective method for analyzing large concentration of the cells in short time (Lee *et.al*.; 2012). However, PAM measurements can be performed in situ, and are not destructive, allowing repetitive measurements along the exposure time, whereas flow cytometry requires the zooxanthellae to be extracted from the coral tissue.

Table 4: Combination of PAM, flow cytometry, and haemocytometry

PAM methods	**Flow cytometry**	**Haemocytometry**
PAM fluorometry gives overall photosynthetic activity of coral tissue PAM can be performed in situ, and are not destructive allowing repetitive measurements along the exposure time.	More accurate and efficient as it takes into account the fluorescence of photosynthetic pigments to differentiate between healthy and unhealthy cells. Effective method for analyzing large concentration of the cells in short time (Lee *et al*., 2012). Requires the zooxanthellae to be extracted from the coral tissue.	Quick, simple and inexpensive Gives the density of the zooxanthellae cell per cm^2

CHAPTER 6: CONCLUSION

Temperature stress is considered as one of the biggest threats for coral health as it negatively affects the photosynthetic activity of the zooxanthellae, which live in symbiotic relationship within the coral tissue. Using diving PAM fluorometry and flow cytometry, we identified the response of the coral to different thermal stress range. Long term exposure to high temperature were noticeably found to affect the photosynthetic process of the zooxanthellae of *Porites lutea* and *Dipsastraea pallida. Acropora downingi,* which is known to be a sensitive species, was very susceptible to high temperatures and was not able to tolerate high temperatures for a long period. This may explain the differential mortality rates noticed between different coral species occurring in a same natural reef. Due to the different responses from different species, maintaining the biodiversity of coral communities is very important and critical for coral growth and competition. This biodiversity can guarantee some survival of the sensitive species after heat stress events.

Coral reef conservation in the Arabian Gulf is vital to ensure economic, social and environmental sustainability of these key ecosystems. Given the already extreme conditions corals are subjected to in the region, coral conservation and restoration should be a priority in the Arabian Gulf

REFERENCES

Baker, A. C. (2003). Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of Symbiodinium. *Annual Review of Ecology, Evolution, and Systematics, 34*(1), 661-689. doi:10.1146/annurev.ecolsys.34.011802.132417

Baker, A. C., Glynn, P. W., & Riegl, B. (2008). Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuarine, Coastal and Shelf Science, 80*(4), 435-471.

Baker, A. C., Starger, C. J., Mcclanahan, T. R., & Glynn, P. W. (2004). Coral reefs: Corals' adaptive response to climate change. *Nature, 430*(7001), 741-741. doi:10.1038/430741a

Belshe, E., Durako, M., & Blum, J. (2007). Photosynthetic rapid light curves (RLC) of Thalassia testudinum exhibit diurnal variation. *Journal of Experimental Marine Biology and Ecology, 342*(2), 253-268. doi:10.1016/j.jembe.2006.10.056

Berkelmans, R., & Oppen, M. J. (2006). The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences, 273*(1599), 2305-2312. doi:10.1098/rspb.2006.3567

Bridge, T. C., Fabricius, K. E., Bongaerts, P., Wallace, C. C., Muir, P. R., Done, T. J., & Webster, J. M. (2011). Diversity of Scleractinia and Octocorallia in the mesophotic zone of the Great Barrier Reef, Australia. *Coral Reefs, 31*(1), 179-189. doi:10.1007/s00338-011-0828-1

Browne, N. K., Tay, J. K., Low, J., Larson, O., & Todd, P. A. (2015). Fluctuations in coral health of four common inshore reef corals in response to seasonal and anthropogenic changes in water quality. *Marine Environmental Research, 105*, 39-52. doi:10.1016/j.marenvres.2015.02.002

Burt, J. A., Al-Khalifa, K., Khalaf, E., Alshuwaikh, B., & Abdulwahab, A. (2013). The continuing decline of coral reefs in Bahrain. *Marine Pollution Bulletin, 72*(2), 357-363. doi:10.1016/j.marpolbul.2012.08.022

Burt, J., Al-Harthi, S., & Al-Cibahy, A. (2011). Long-term impacts of coral bleaching events on the world's warmest reefs. *Marine Environmental Research, 72*(4), 225-229. doi:10.1016/j.marenvres.2011.08.005

Burt, J., Bartholomew, A., & Usseglio, P. (2008). Recovery of corals a decade after a bleaching event in Dubai, United Arab Emirates. *Marine Biology, 154*(1), 27-36. doi:10.1007/s00227-007-0892-9

Burt, J. A., Smith, E. G., Warren, C., & Dupont, J. (2016). An assessment of Qatar's coral communities in a regional context. *Marine Pollution Bulletin, 105*(2), 473-479. doi:10.1016/j.marpolbul.2015.09.025

Caldeira, K. & Wickett, M. E. (2003). Anthropogenic carbon and ocean pH. *Nature* 425, 365

Coles, S. L., & Riegl, B. M. (2013). Thermal tolerances of reef corals in the Gulf: A review of the potential for increasing coral survival and adaptation to climate change through assisted translocation. *Marine Pollution Bulletin, 72*(2), 323-332. doi:10.1016/j.marpolbul.2012.09.006

Douglas, A. E. (1994). Symbiotic Interactions. Oxford: Oxford Science Publications.

Douglas, A. E. (2003). Coral bleaching—how and why?. *Marine Pollution Bulletin, 46*(4), 385-392.

El Nino Theme Page - A comprehensive Resource - pmel.noaa.gov. (2007). Retrieved April 9, 2017, from http://www.pmel.noaa.gov/tao/elnino/el-nino-story.html

Furby, K. A., Bouwmeester, J., & Berumen, M. L. (2013). Susceptibility of central Red Sea corals during a major bleaching event. *Coral Reefs, 32*(2), 505-513. doi:10.1007/s00338-012-0998-5.

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

Fitt, W. K., Mcfarland, F. K., Warner, M. E., & Chilcoat, G. C. (2000). Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnology and Oceanography, 45*(3), 677-685. doi:10.4319/lo.2000.45.3.0677

Gates, R. D., & Edmunds, P. J. (1999). The physiological mechanisms of acclimatization in tropical reef corals. *American Zoologist, 39*(1), 30-43

Global Marine Species Assessment (2008). Retrieved Mar 2, 2017, from http://www.sci.odu.edu/gmsa/

Goreau, T. F. (2006). Calcium Carbonate Deposition By Coralline Algae And Corals In Relation To Their Roles As Reef-Builders. *Annals of the New York Academy of Sciences, 109*(1), 127-167

Hagedorn, M., Carter, V., Leong, J., Kleinhans, F. (2010) Physiology and cryosensitivity of coral endosymbiotic algae (Symbiodinium). *Cryobiology* 60:147-158

Hallock, P. (2001). Coral Reefs, Carbonate Sediments, Nutrients, and Global Change. *Topics in Geobiology The History and Sedimentology of Ancient Reef Systems,* 387-427. doi:10.1007/978-1-4615-1219-6_11

Howells, E. J., Abrego, D., Meyer, E., Kirk, N. L., & Burt, J. A. (2016). Host adaptation and unexpected symbiont partners enable reef-building corals to tolerate extreme temperatures. *Global Change Biology, 22*(8), 2702-2714. doi:10.1111/gcb.13250

Howells, E. J., Beltran, V. H., Larsen, N. W., Bay, L. K., Willis, B. L., & Oppen, M. J. (2011). Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change, 2*(2), 116-120. doi:10.1038/nclimate1330

Howells, E. J., Berkelmans, R., van Oppen, M. J. H., Willis, B. L. and Bay, L. K. (2013), Historical thermal regimes define limits to coral acclimatization. *Ecology*, 94: 1078–1088. doi:10.1890/12-1257.1

Howells, E. J., Ketchum, R. N., Bauman, A. G., Mustafa, Y., Watkins, K. D., & Burt, J. A. (2016). Species-specific trends in the reproductive output of corals across environmental gradients and bleaching histories. *Marine Pollution Bulletin, 105*(2), 532-539. doi:10.1016/j.marpolbul.2015.11.034

Hume, B. C., D'angelo, C., Smith, E. G., Stevens, J. R., Burt, J., & Wiedenmann, J. (2015). Symbiodinium thermophilum sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. *Scientific Reports, 5*, 8562. doi:10.1038/srep08562

Jackson, J. B. (2001). Historical Overfishing and the Recent Collapse of Coastal Ecosystems. *Science, 293*(5530), 629-637. doi:10.1126/science.1059199

Johnson, C. R., Dunstan, P. K., Hoegh-Guldberg, O. (2002). Predicting the long term effects of coral bleaching and climate change on the structure of coral communities. *NCORE abstracts*, Miami, USA

Jones, G., & King, S. (2015). Dimethylsulphoniopropionate (DMSP) as an Indicator of Bleaching Tolerance in Scleractinian Corals. *Journal of Marine Science and Engineering, 3*(2), 444-465. doi:10.3390/jmse3020444

Kemp, D. W., Hernandez-Pech, X., Iglesias-Prieto, R., Fitt, W. K., & Schmidt, G. W. (2014). Community dynamics and physiology of Symbiodiniums pp. before, during, and after a coral bleaching event. *Limnology and Oceanography, 59*(3), 788-797. doi:10.4319/lo.2014.59.3.0788

Krediet, C. J., Denofrio, J. C., Caruso, C., Burriesci, M. S., Cella, K., & Pringle, J. R. (2015). Rapid, Precise, and Accurate Counts of Symbiodinium Cells Using the Guava Flow Cytometer, and a Comparison to Other Methods. *Plos One, 10*(8). doi:10.1371/journal.pone.0135725

Krediet, C. J., Ritchie, K. B., Paul, V. J., & Teplitski, M. (2013). Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. *Proceedings of the Royal Society B: Biological Sciences, 280*(1755), 20122328-20122328. doi:10.1098/rspb.2012.2328

Lee, C. S., Yeo, Y. S., & Sin, T. M. (2012). Bleaching response of Symbiodinium (zooxanthellae): Determination by flow cytometry. *Cytometry Part A, 81A*(10), 888-895. doi:10.1002/cyto.a.22111

Lough, J., Barnes, D., & McAllister, F. (2002). Luminescent lines in corals from the Great Barrier Reef provide spatial and temporal records of reefs affected by land runoff. *Coral Reefs , 21*(333). doi:10.1007/s00338-002-0253-6

Miththapala, S. (2008). *Coral Reefs "Coastal ecosystems series"*. Colombo: Ecosystems and Livelihoods Group Asia, IUCN.

Moberg, F., & Folke, C. (1999). Ecological goods and services of coral reef ecosystems. *Ecological Economics, 29*(2), 215-233. doi:10.1016/s0921-8009(99)00009-9

Muscatine, L., & Porter, J. W. (1977). Reef Corals: Mutualistic Symbioses Adapted to Nutrient-Poor Environments. *BioScience, 27*(7), 454-460. doi:10.2307/1297526

Pilcher, N., Wilson, S., Alhazeem, S., & Shokri, M. (2000). Status of coral reefs in the Arabian/Persian gulf and Arabian Sea. *Australian Institute of Marine science,* 55-64

Rädecker, N., Pogoreutz, C., Voolstra, C. R., Wiedenmann, J., & Wild, C. (2015). Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends in Microbiology, 23*(8), 490-497. doi:10.1016/j.tim.2015.03.008

Richier, S., Furla, P., Plantivaux, A., Merle, P.-L. and Allemand, D. (2005). Symbiosis-induced adaptation to oxidative stress. *J. Exp. Biol.* 208, 277-285.

Riegl, B. (2002). Effects of the 1996 and 1998 positive sea-surface temperature anomalies on corals, coral diseases and fish in the Arabian Gulf (Dubai, UAE). *Marine Biology, 140*(1), 29-40. doi:10.1007/s002270100676

Riegl, B. M., & Purkis, S. J. (2012). Coral Reefs of the Gulf: Adaptation to Climatic Extremes in the World's Hottest Sea. *Coral Reefs of the World Coral Reefs of the Gulf,* 1-4. doi:10.1007/978-94-007-3008-3_1

Salih, A., Hoegh-Guldberg, Guy C. (2006). Bleaching Responses of Symbiotic Dinoflagellates in Corals: The Effects of Light and Elevated Temperature on their Morphology and Physiology

Saxena, A. (2015). Coral Reefs and Their Conservation — A Review. *Biological and Chemical Research,* 187-206.

SCENR (Supreme Council for Environment and Natural Reserves of Qatar). (2007).

Schnitzler, C. E., & Weis, V. M. (2010). Coral larvae exhibit few measurable transcriptional changes during the onset of coral-dinoflagellate endosymbiosis. *Marine Genomics, 3*(2), 107-116. doi:10.1016/j.margen.2010.08.002

Sheppard, C. (2016). Coral reefs in the Gulf are mostly dead now, but can we do anything about it?. *Marine Pollution Bulletin,* 105:593-598

Sheppard, C., Al-Husiani, M., Al-Jamali, F., Al-Yamani, F., Baldwin, R., Bishop, J., Benzoni, F., Dutrieux, E., Dulvy, N.K., Durvasula, S.R.V. (2012). Environmental

concerns for the future of Gulf coral reefs Coral Reefs of the Gulf. *Springer,* 349-373

Sheppard, C. R. (1993). Physical environment of the Gulf relevant to marine pollution: An overview. *Marine Pollution Bulletin, 27*, 3-8. doi:10.1016/0025-326x(93)90003

Shuail, D., Wiedenmann, J., D'Angelo, C., Baird, A.H., Pratchett, M.S., Riegl, B., Burt, J.A., Petrov, P., Amos, C. (2016). Local bleaching thresholds established by remote sensing techniques vary among reefs with deviating bleaching patterns during the 2012 event in the Arabian/Persian Gulf. *Marine Pollution Bulletin*, 105:654-659

Stat, M., Morris, E., & Gates, R. D. (2008). Functional diversity in coral-dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences, 105*(27), 9256-9261. doi:10.1073/pnas.0801328105

Sumich, J. L. (1996). *Introduction to the biology of marine life*. Jones & Bartlett Learning.

Taher, M. M., Mohamed, A. R., & Al-Ali, A. K. (2012). Some ecological characteristics and ichthyofauna of surrounding Sammaliah Island, Abu Dhabi, UAE. *Basrah Journal of Science, 30*(2), 31-49.

Ulstrup, K. E. & van Oppen, M. J. H. (2003). Geographic and habitat partitioning of genetically distinct zooxanthellae (Symbiodinium) in Acropora corals on the Great Barrier Reef. *Mol. Ecol*. 12, 3477–3484. (doi:10.1046/j.1365-294X.2003.01988.x)

Veron, J. E. N. (2000). Corals of the World, vol. 3. *Australian Institute of Marine Science & CRR Qld Pty Ltd, Australia*

Vitousek, P. M. (1994). Beyond Global Warming: Ecology and Global Change. *Ecology, 75*(7), 1861-1876. doi:10.2307/1941591

Vogt, H.P. & K. Al-Shaikh, (2000). Status of Saudi Arabian coral reefs in the Arabian Gulf following coral bleaching in 1997/1998. *In Proceedings of the International Workshop on the Extent and Impact of Coral Bleaching in the Arabian Region.* National Commission for Wildlife Conservation and Development, Riyadh

Wild, C., Hoegh-Guldberg, O., Naumann, M. S., Colombo-Pallotta, M. F., Ateweberhan, M., Fitt, W. K., R., Iglesias-Prieto, Palmer, C., Bythell, J.C., Ortiz, J.-C., Loya, Y. Woesik, R. V. (2013). Climate change impedes scleractinian corals as primary reef ecosystem engineers. *Marine and Freshwater Research, 62*(2), 205. doi:10.1071/mf10254

Wooldridge, S. A. (2012). Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. *Biogeosciences Discussions, 9*(7), 8111-8139. doi:10.5194/bgd-9-8111-2012

Wilkison, C. (1998). The 1997-1998 Mass Bleaching Event around the World. *OceanDocs*

Yellowlees, D., Rees, T. A. V. and Leggat, W. (2008). Metabolic interactions between algal symbionts and invertebrate hosts. *Plant Cell Environ.* 31, 679-694.

APPENDIX A: WATER QUALITY PARAMETERS AND FIGURES

Water quality parameters:

Salinity (psu)

Date	1A	1B	1C	2A	2B	2C
24-Jan	41	40	40	40	42	41
26-Jan	45	42	42	42	41	42
29-Jan	42	41	42	42	42	41
31-Jan	42	42	41	42	43	42
5-Feb	43	42	41	42	43	42
6-Feb	43	42	42	42	43	43
12-Feb	42	42	41	42	42	42
13-Feb	41	42	41	41	42	42
15-Feb	42	41	42	41	42	41
16-Feb	41	41	41	41	42	42
19-Feb	41	41	42	41	42	41
20-Feb	41	42	41	43	43	42
21-Feb	41	41	42	41	42	41
26-Feb	41	42	41	42	42	43
27-Feb	41	41	41	42	41	41
1-Mar	42	41	42	42	42	41
2-Mar	41	41	40	41	41	41
5-Mar	42	41	41	43	41	41
6-Mar	41	41	41	42	41	41
7-Mar	41	42	42	42	42	41
8-Mar	43	42	40	41	40	40
13-Mar	41	42	41	41	41	40
15-Mar	41	41	40	41	41	41
20-Mar	41	41	41	41	41	42
29-Mar	41	41	41	41	41	40

Temperature °C

Date	1A	1B	1C	2A	2B	2C
24-Jan	27.6	27.1	24.1	25	28.1	27.3
26-Jan	23.5	23.2	23.1	23.6	23.4	23.1
29-Jan	23.5	23.1	22.3	23	22.9	22.8
31-Jan	23.8	23.4	22.7	23.1	23	22.9
5-Feb	23.4	23	22.5	22.6	22.6	22.5
12-Feb	23.7	23.3	23	23.2	23.2	23.2
15-Feb	26.9	26.9	24.9	24.1	24.1	24.3
19-Feb	28.3	28.2	25.3	24		24.5
21-Feb	27.3	28.3	25	24.8	27.1	27.3
22-Feb	29.8	29.3	25.4	25.9	28.7	28.7
23-Feb	29.3	29.7	25.7	25.7	28.4	28.3
26-Feb	28.7	28.1	25.3	25.2	28.7	28.8
27-Feb	29.2	28.8	25.5	25.3	28	29.2
1-Mar	30.1	28.5	25.4	25.6	28.4	30.6
2-Mar	33.2	30.9	26.6	26.8	32.1	34.1
5-Mar	33.5	31.7	26.6	26.9	32.3	34.8
6-Mar	35.7	32.3	26.6	26.6	31.4	33
15-Mar	35.7	31.9	27.1	26.9	32	35.5
20-Mar	35.7	31.4	26.7	27	33.1	35.6
21-Mar	35.7	31.6	26.4	27.4	33	35.5
26-Mar	32.7	31.3	26.3	26.1	31.7	32.7
29-Mar	32.6	30.5	26.3	26.2	31.4	31.5

pH

Date	1A	1B	1C	2A	2B	2C
29-Jan	7.79	7.89	7.96	8.02	8.03	8.01
31-Jan	7.88	7.86	8.03	7.95	7.98	7.94
5-Feb	8.08	8.13	7.98	8	8.03	8.02
6-Feb	7.98	8	7.94	8.02	8.05	7.93
7-Feb	8.04	8.01	7.91	7.98	7.98	7.88
9-Feb	7.94	7.95	7.94	7.95	7.98	7.84
12-Feb	7.99	7.99	7.99	7.94	7.99	7.89
15-Feb	7.98	7.97	7.98	7.95	8.04	7.77
19-Feb	7.9	8.01	8.05	7.94	8.03	7.89
21-Feb	7.93	7.88	7.95	7.84	7.93	7.73
22-Feb	7.94	7.96	7.97	7.87	7.96	7.78
26-Feb	7.99	8.02	8.05	7.9	7.93	7.96
27-Feb	7.87	7.82	7.86	7.87	7.9	7.88
1-Mar	7.92	7.88	7.9	7.96	7.94	7.89
2-Mar	7.8	7.75	7.81	7.81	7.81	7.71
6-Mar	7.93	7.9	7.95	7.98	8	7.92
12-Mar	8.09	8.04	8.04	8.03	8.07	7.89

20-Mar	7.99	7.92	8.09	8.03	8.1	8.02
29-Mar	8.01	8.03	8.09	8.03	8.1	7.99

NO3 mg/l (ppm)

Date	1A	1B	1C	2A	2B	2C
29-Jan	0.75	0.75	0.75	0.75	1	0.75
31-Jan	0	0	0	0	0	0
5-Feb	0	0	0	0	0	0
12-Feb	0	0	0	0	0	0
19-Feb	0	0	0	0	0	0
27-Feb	0	0	0	0	0	0
6-Mar	0	0	0	0	0	0
7-Mar						0
8-Mar						0
12-Mar	0	0	0	0	0	5
13-Mar	0	0				5
19-Mar	1	0	0	0	0	1
20-Mar	0.75	0				0
26-Mar	0	0	0	0	0.75	0
29-Mar	0	1			0	0

NO2 mg/l (ppm)

Date	1A	1B	1C	2A	2B	2C
29-Jan	0.05	0.5	0.15	0.15	0.3	0.15
31-Jan	0.05	0.5	0.05	0.5	0.1	0.05
5-Feb	0.05	0.04	0.05	0.05	0.5	0.5
6-Feb	0.04	0.05	0.04	0.05	0.04	0.04
12-Feb	0.04	0.05	0.025	0.025	0.05	0.04
15-Feb	0.025	0.04	0.025	0.05	0.05	0.025
19-Feb	0.025	0.025	0.05	0.025	0.1	0.025
21-Feb	0.025	0.025	0.05	0.025	0.1	0.05
26-Feb	0.025	0.025	0.025	0.025	0.05	0.05
1-Mar	0.025	0.025	0.025	0.025	0.05	0.05
5-Mar						0.4
6-Mar	0.05	0.05	0.025	<0.01	0.05	0.4
7-Mar						0.4
8-Mar						0.2
12-Mar	0.3	0.1	0.025	0.025	0.05	0.6
13-Mar	0.2	0.05				0.6
19-Mar	0.2	0.1	< 0.01	< 0.01	< 0.01	0.2
20-Mar	0.2	0.1				0.2
26-Mar	0.4	0.4	< 0.01	< 0.01	0.2	0.1
29-Mar	0.4	0.6			0.2	0.1

PO4 (mg/l)

Date	**1A**	**1B**	**1C**	**2A**	**2B**	**2C**
29-Jan	**0.05**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
5-Feb	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
15-Feb	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
19-Feb	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
27-Feb	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
6-Mar	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
7-Mar	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
12-Mar	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
20-Mar	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**
29-Mar	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**

NH4 (mg/l)

Date	1A	1B	1C	2A	2B	2C
29-Jan	0.05	0.05	0.05	0.05	0.05	0.05
31-Jan	0.05	0.05	0.05	0.05	0.05	0.05
5-Feb	0.05	0.05	0.05	0.05	0.05	0.05
12-Feb	0.05	0.05	0.05	0.05	0.05	0.05
19-Feb	0.05	0.05	0.05	0.05	0.05	0.05
27-Feb	0.05	0.05	0.05	0.05	0.05	0.05
6-Mar	0.1	0.1	0.05	0.05	0.1	0.6
7-Mar						0.4
12-Mar	0.2	0.1	0.05	0.05	0.05	0.4
19-Mar	0.4	0.05	0.05	0.05	0.05	0.05
20-Mar	0.2	0.1				0.05
26-Mar	0.05	0.1	0.05	0.05	0.05	0.05
29-Mar	0.05	0.05			0.05	0.05

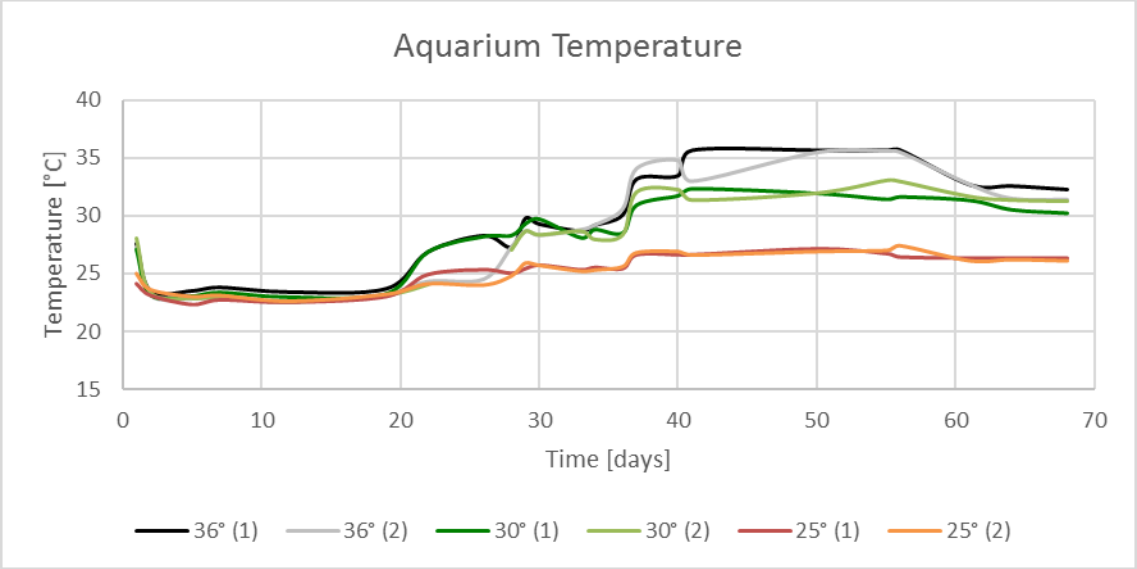
CA (mg/l)

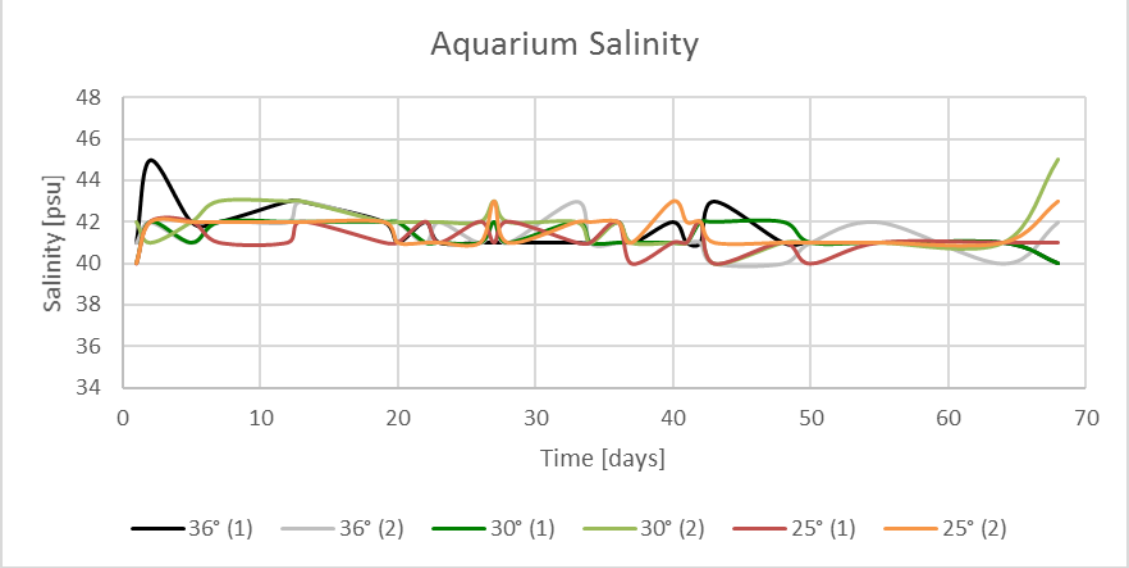
Date	1A	1B	1C	2A	2B	2C
29-Jan	520	600	560	600	680	680
5-Feb	520	560	520	640	620	580
15-Feb	560	580	540	580	620	580
21-Feb	450	480	500	600	520	410
23-Feb				375	387	325
26-Feb	425	375	450	400	410	365
6-Mar	360	650	550	400	500	450
13-Mar	375	580	500	580	520	360
20-Mar	355	425	560	400	480	325

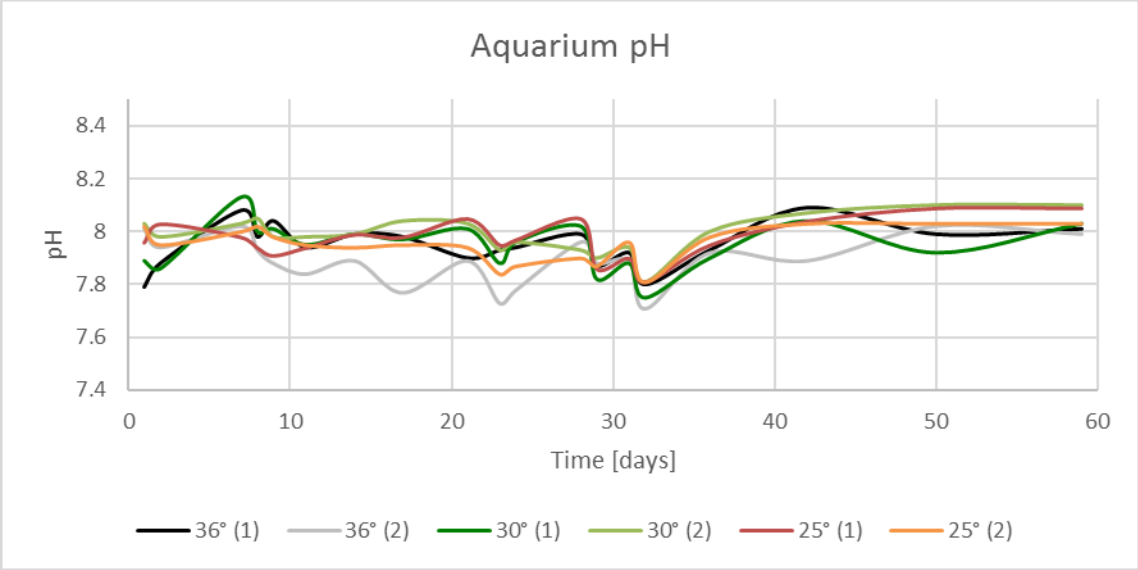
Mg (mg/l)

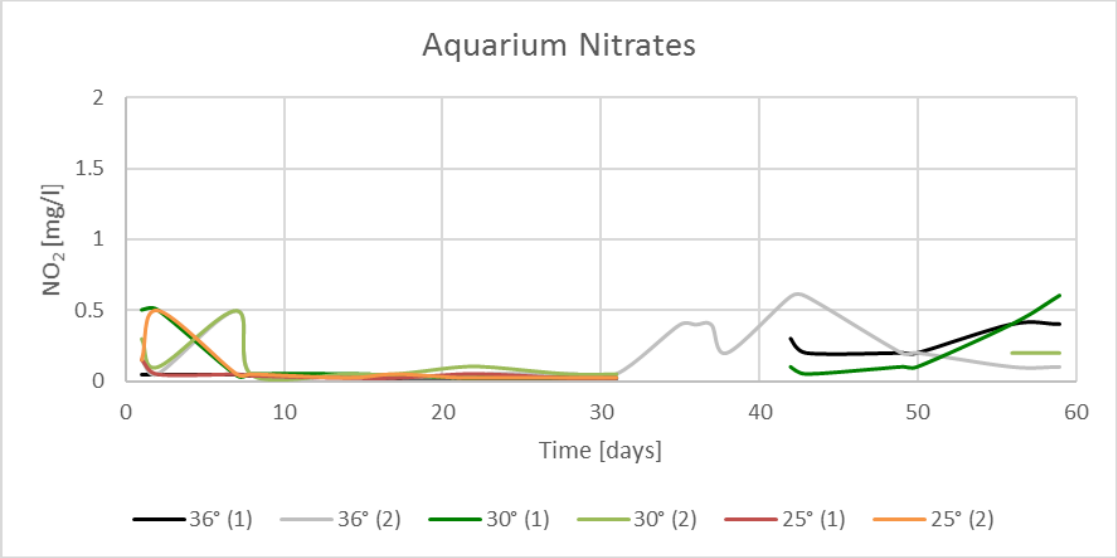
Date	1A	1B	1C	2A	2B	2C
29-Jan	1780	1600	1440	1500	1320	1120
5-Feb	1781	1540	980	1160	1280	1122
15-Feb	1240	1220	1120	1120	1180	1220
21-Feb	1275	1250	1250	1375	1200	1380.5
27-Feb	1200	1275	1125	1375	1125	1200
6-Mar	1750	1500	962	1375	1250	1024
13-Mar	1275	1450	1200	1125	1200	1125
20-Mar	1275	1250	1125	1120	1124	1036

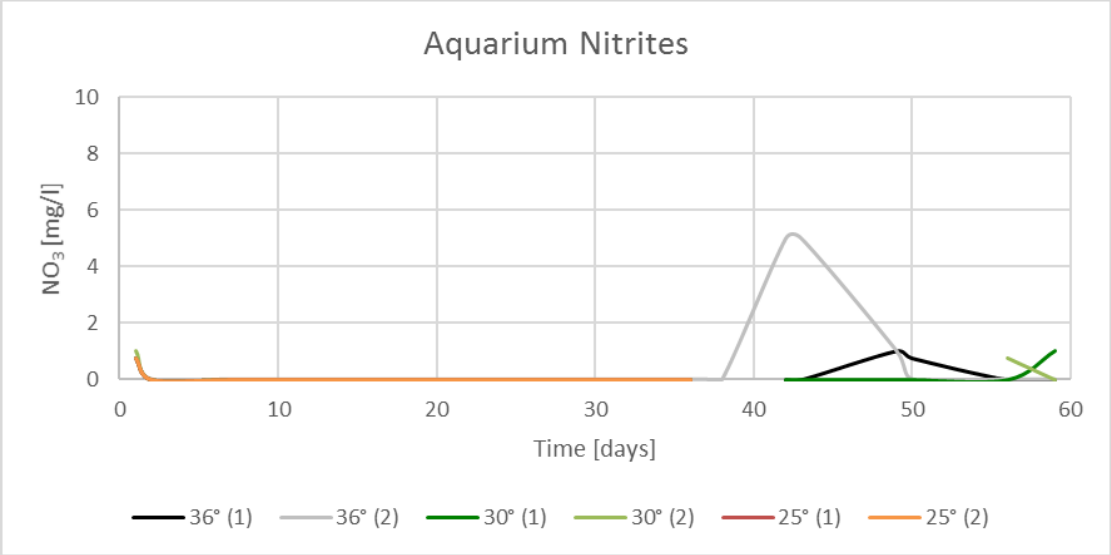
Water Chemistry Figures:

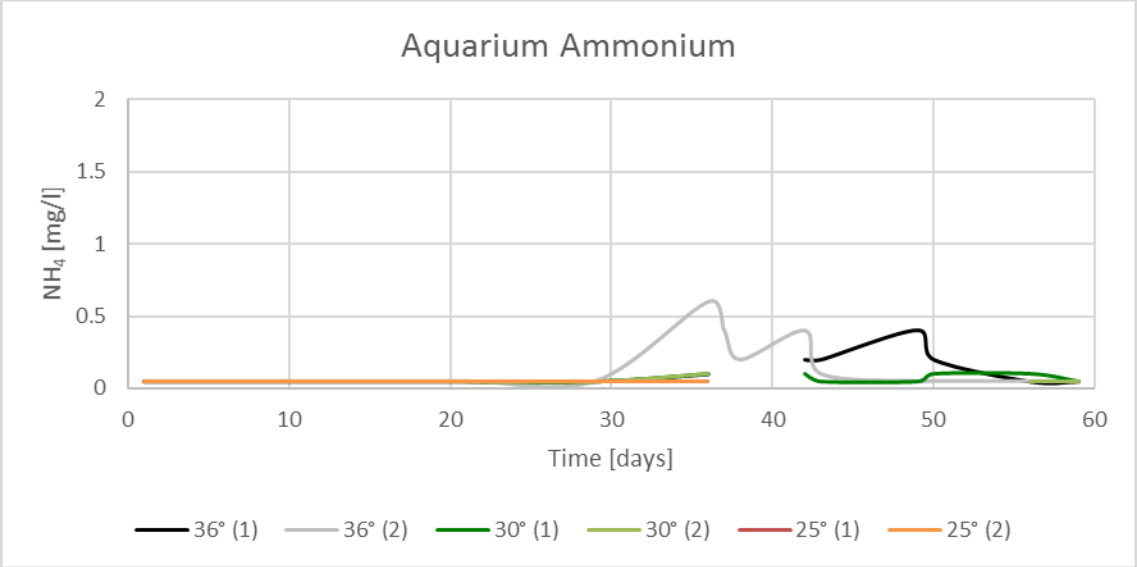


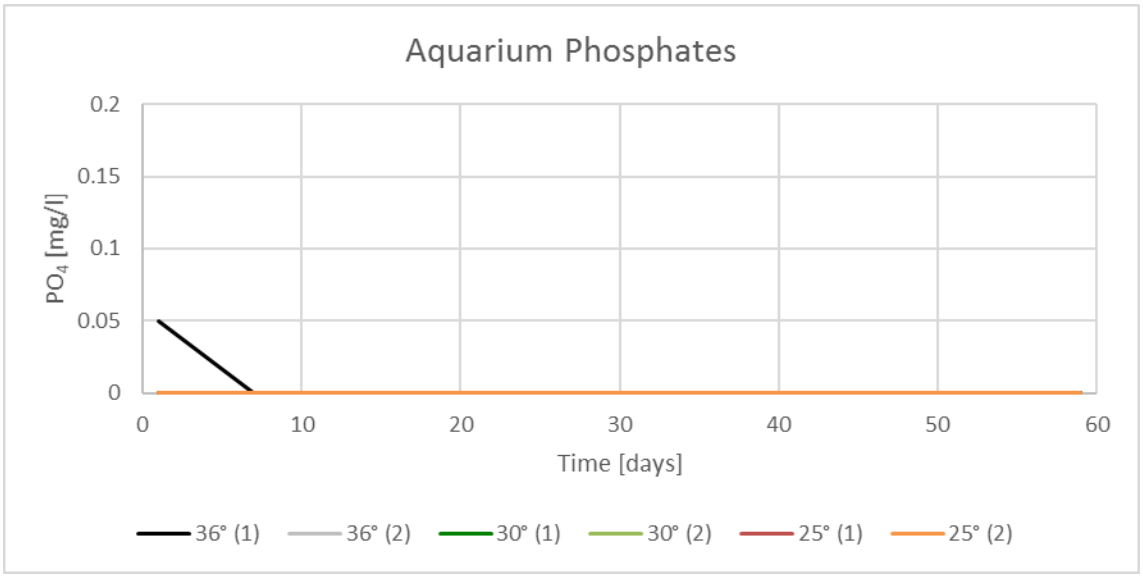


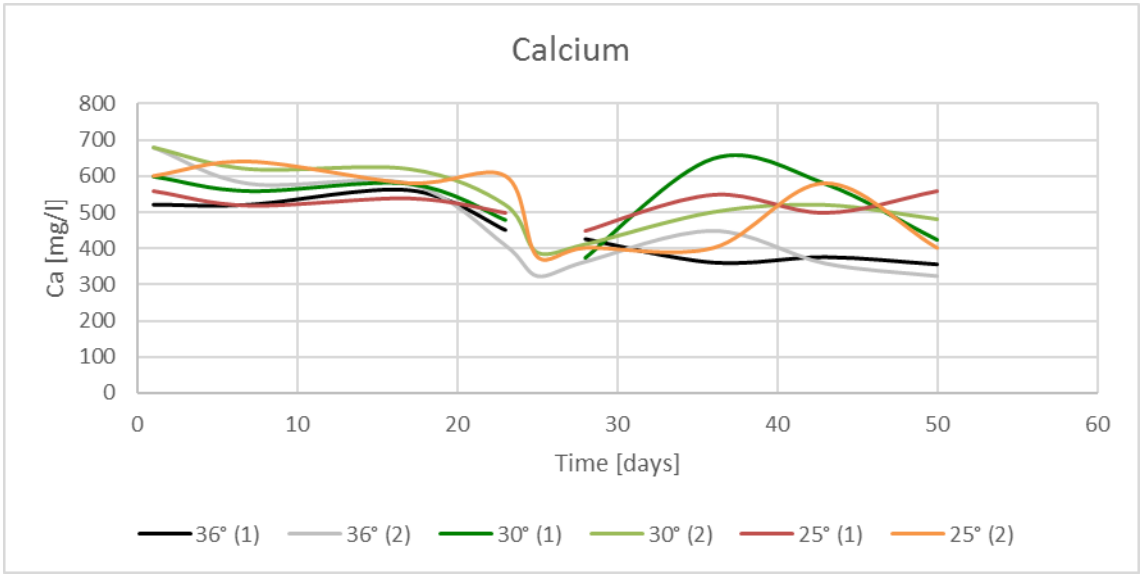

Aquarium pH
8.4
8.2
8
7.8
7.6
7.4
pH
0
10
20
30
40
50
60
Time [days]
36° (1)
36° (2)
30° (1)
30° (2)
25° (1)
25° (2)

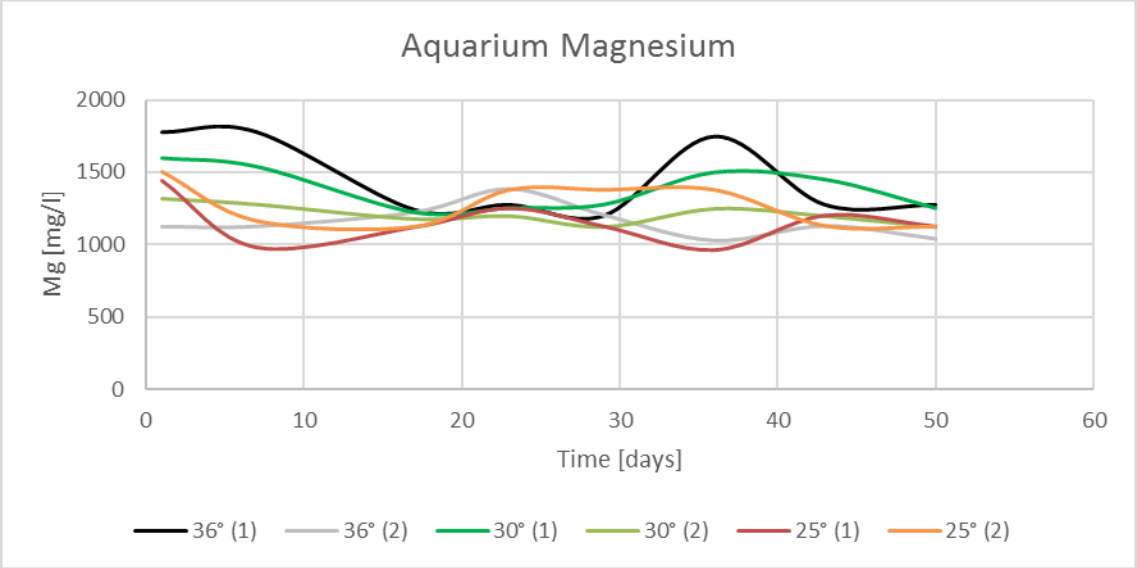

Aquarium Nitrates
2
1.5
1
0.5
0
NO2 [mg/l]
0
10
20
30
40
50
60
Time [days]
36° (1)
36° (2)
30° (1)
30° (2)
25° (1)
25° (2)


Aquarium Nitrites
10
8
6
4
2
0
NO_3 [mg/l]
0
10
20
30
40
50
60
Time [days]
36° (1)
36° (2)
30° (1)
30° (2)
25° (1)
25° (2)


Aquarium Ammonium
2
1.5
1
0.5
0
NH_4 [mg/l]
0
10
20
30
40
50
60
Time [days]
36° (1)
36° (2)
30° (1)
30° (2)
25° (1)
25° (2)


Aquarium Phosphates
0.2
0.15
0.1
0.05
0
PO4 [mg/l]
0
10
20
30
40
50
60
Time [days]
36° (1)
36° (2)
30° (1)
30° (2)
25° (1)
25° (2)


Calcium
800
700
600
500
400
300
200
100
0
Ca [mg/l]
0
10
20
30
40
50
60
Time [days]
36° (1)
36° (2)
30° (1)
30° (2)
25° (1)
25° (2)


Aquarium Magnesium
2000
1500
1000
500
0
Mg [mg/l]
0
10
20
30
40
50
60
Time [days]
36° (1)
36° (2)
30° (1)
30° (2)
25° (1)
25° (2)

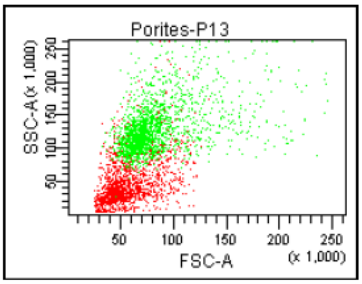
APPENDIX B: FLOW CYTOMETRY ANALYSIS

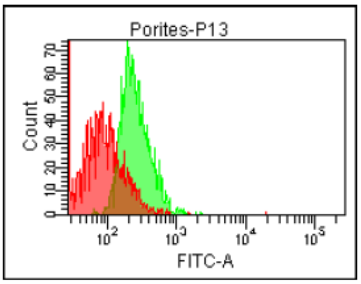
Flow Cytometry Analyses on samples extracted from:
–*Porites lutea* (P)

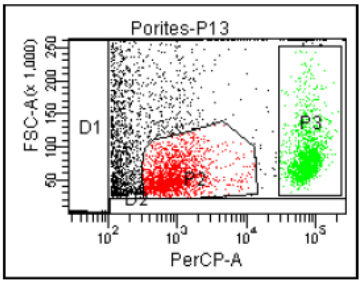
–*Dipsastraea pallida* (D)

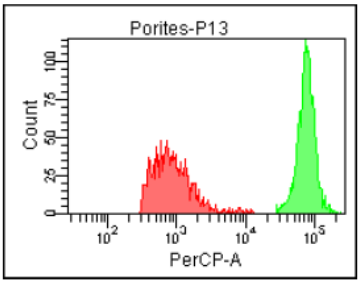
–*Acropora downingi(A)*

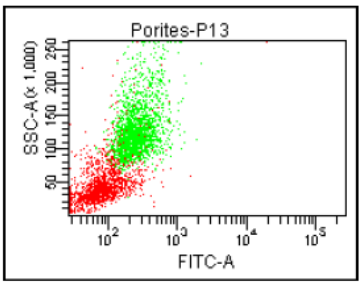
And under the three experimental treatments (25, 30 and 35)

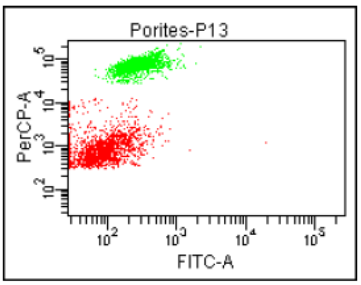






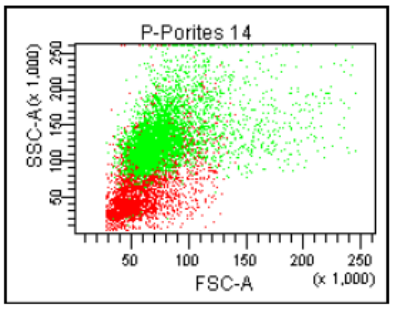


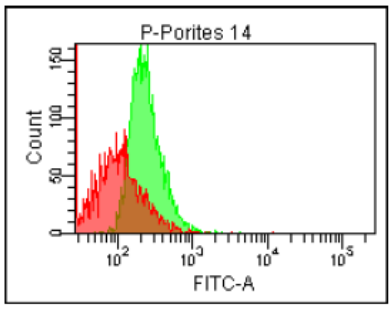


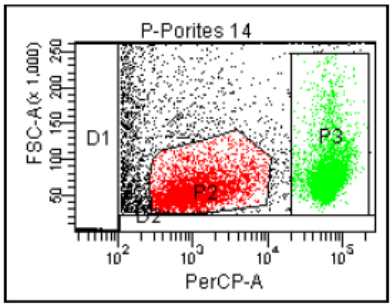


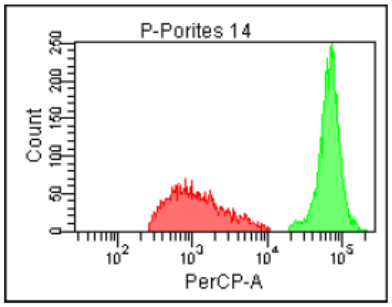
Tube: P13

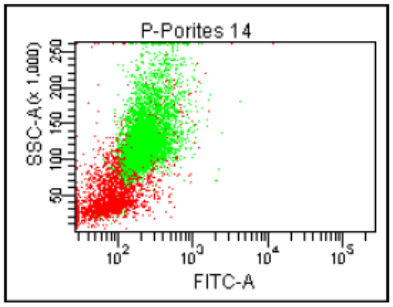
Population	#Events	%Parent	%Total
All Events	359,383	####	100.0
D1	350,277	97.5	97.5
NOT(P1)	9,106	2.5	2.5
P2	1,666	18.3	0.5
P3	1,857	20.4	0.5
D2	1,254	13.8	0.3

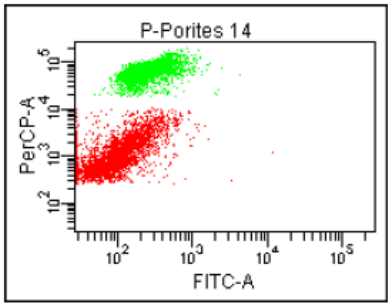






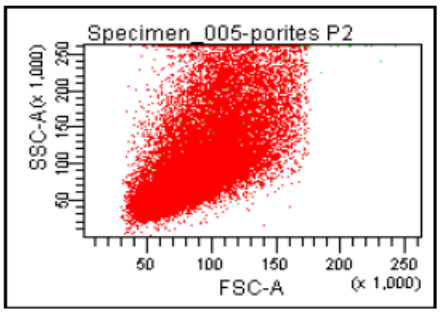


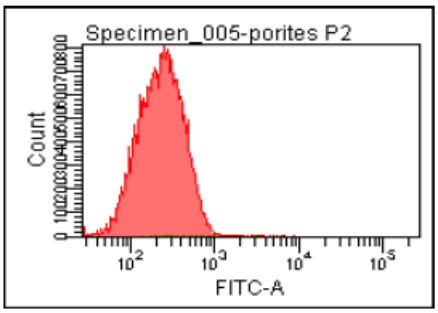


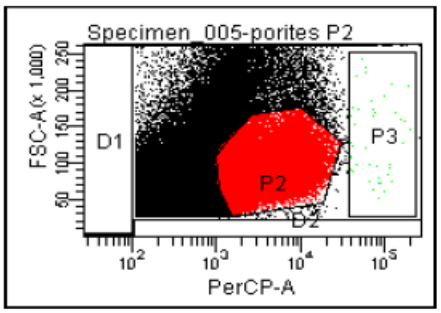


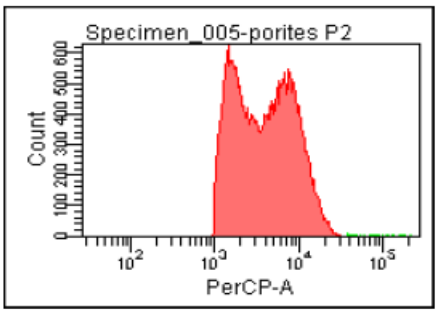
Tube: Porites 14

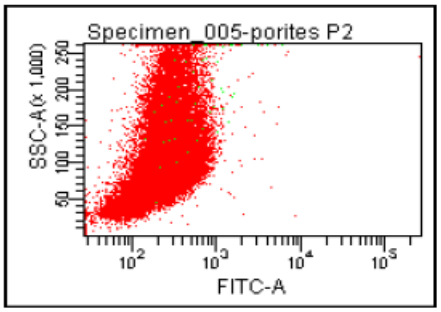
Population	#Events	%Parent	%Total
All Events	502,158	####	100.0
D1	483,987	96.4	96.4
NOT(P1)	18,171	3.6	3.6
P2	3,230	17.8	0.6
P3	4,380	24.1	0.9
D2	1,071	5.9	0.2

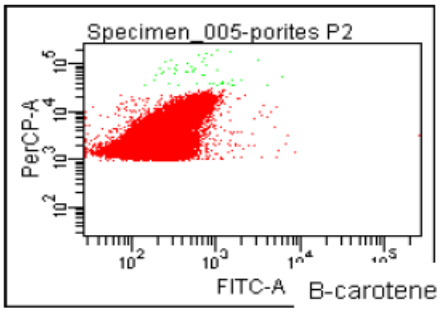






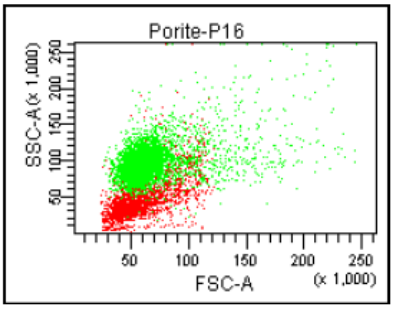


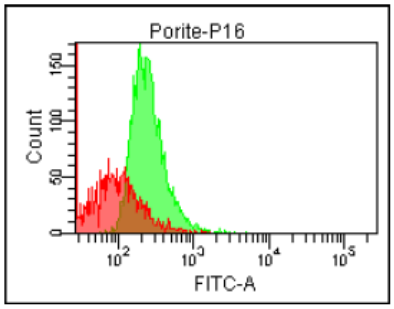


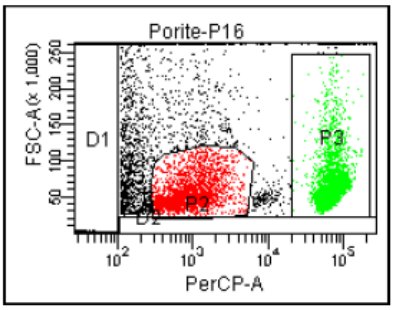


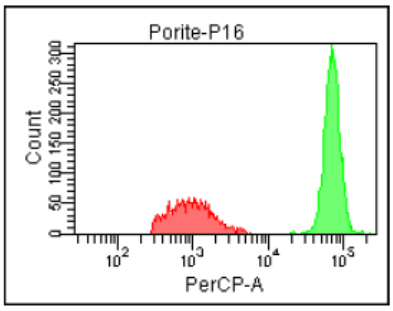
Tube: porites P2

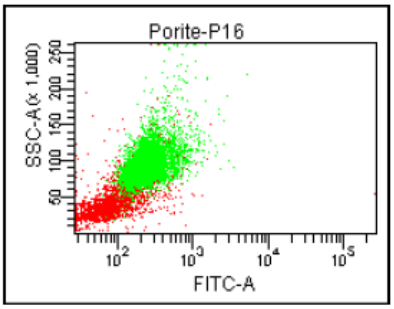
Population	#Events	%Parent	%Total
All Events	590,361	####	100.0
D1	356,505	60.4	60.4
NOT(P1)	233,856	39.6	39.6
P2	32,728	14.0	5.5
P3	49	0.0	0.0
D2	60,755	26.0	10.3

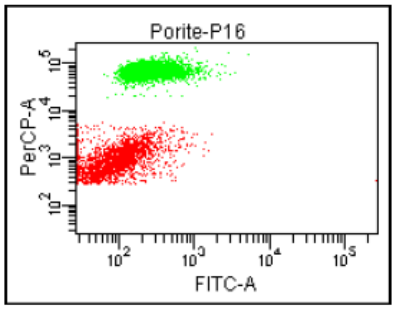






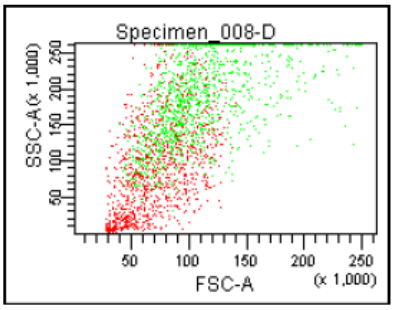


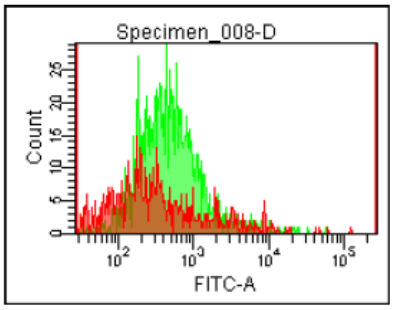


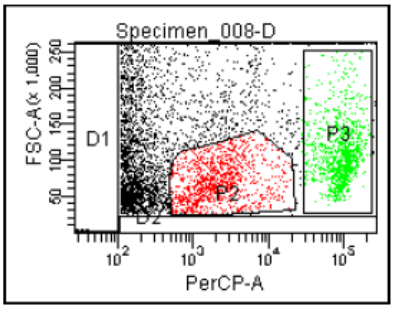


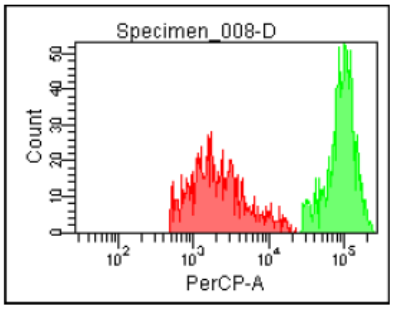
Tube: P16

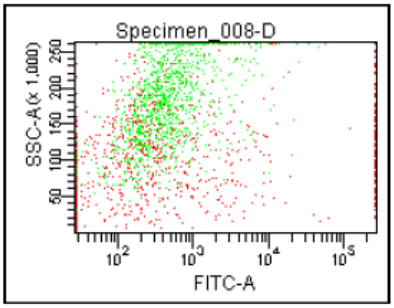
Population	#Events	%Parent	%Total
All Events	277,239	####	100.0
D1	266,331	96.1	96.1
NOT(P1)	10,908	3.9	3.9
P2	2,481	22.7	0.9
P3	4,716	43.2	1.7
D2	713	6.5	0.3

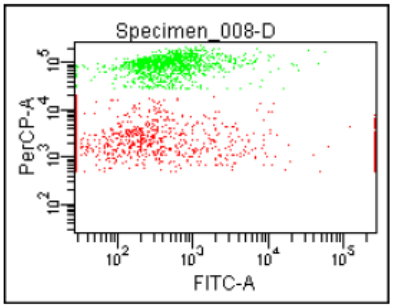






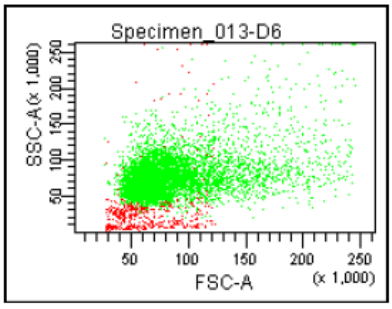


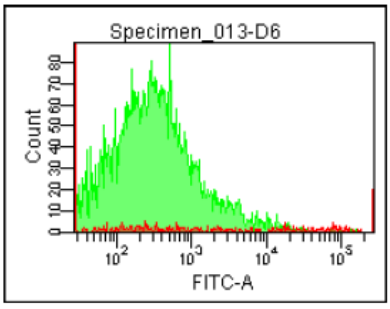


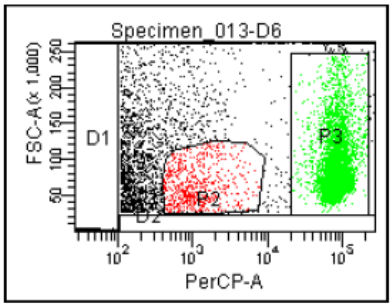


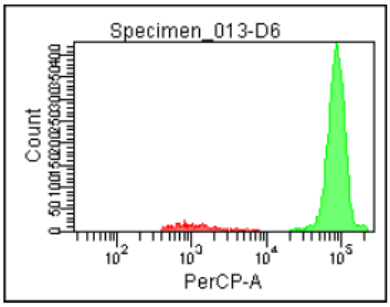
Tube: D

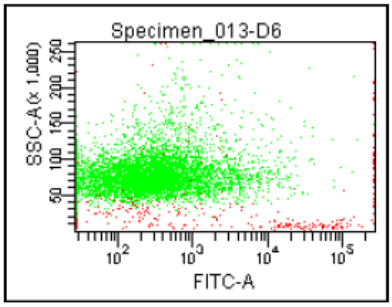
Population	#Events	%Parent	%Total
All Events	528,535	####	100.0
D1	519,040	98.2	98.2
NOT(P1)	9,495	1.8	1.8
P2	1,072	11.3	0.2
P3	1,161	12.2	0.2
D2	1,308	13.8	0.2

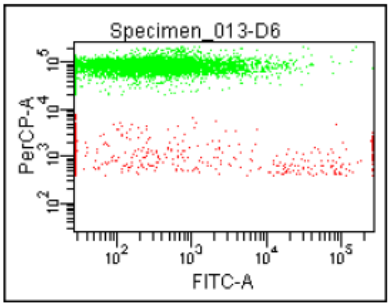






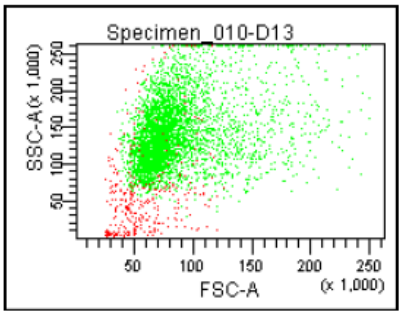


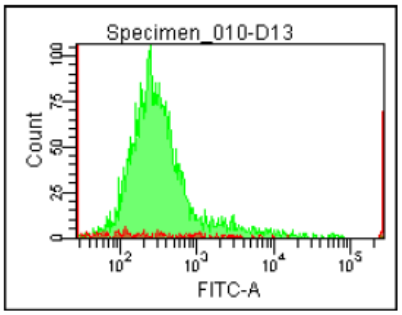


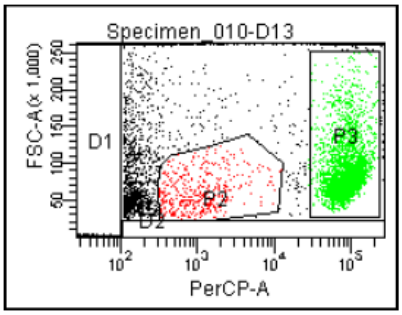


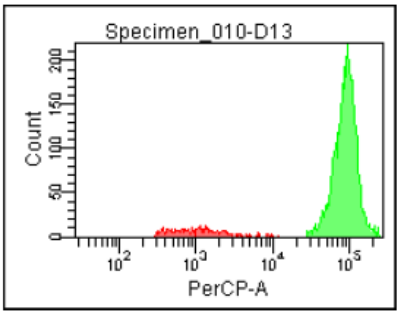
Tube: D6

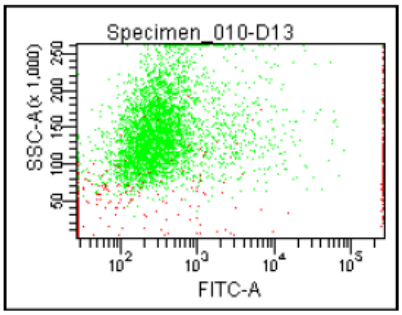
Population	#Events	%Parent	%Total
All Events	663,441	####	100.0
D1	645,397	97.3	97.3
NOT(P1)	18,044	2.7	2.7
P2	597	3.3	0.1
P3	7,081	39.2	1.1
D2	784	4.3	0.1

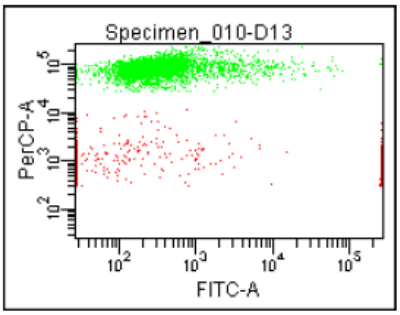






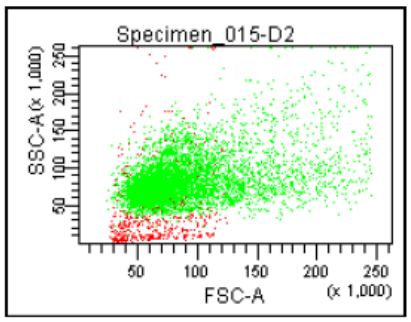


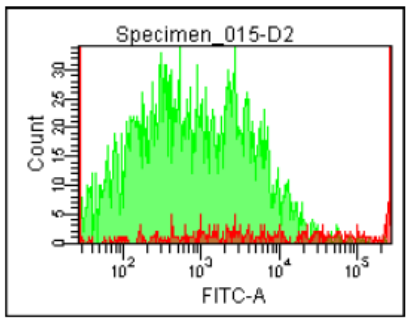


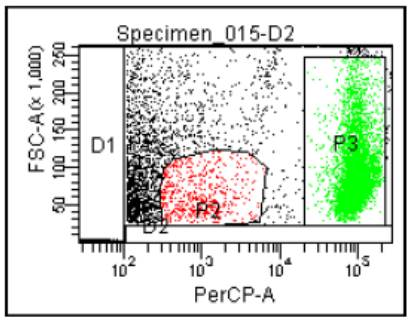


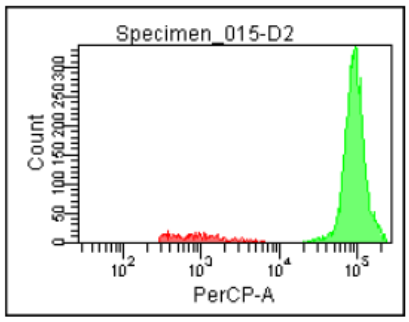
Tube: D13

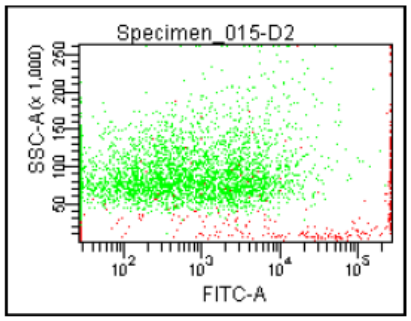
Population	#Events	%Parent	%Total
All Events	1,140,510	####	100.0
D1	1,129,769	99.1	99.1
NOT(P1)	10,741	0.9	0.9
P2	440	4.1	0.0
P3	3,961	36.9	0.3
D2	262	2.4	0.0

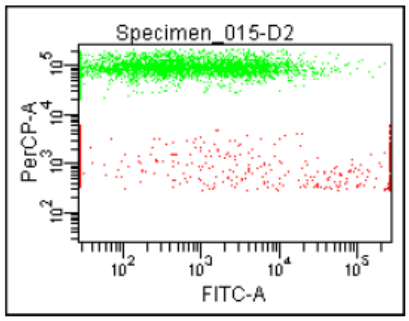






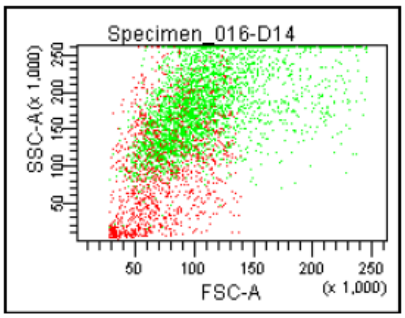


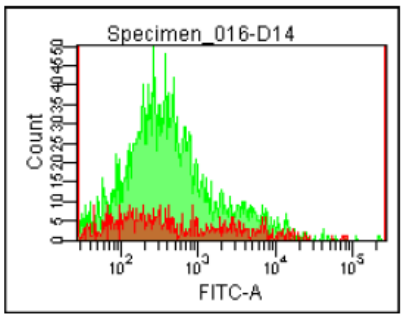


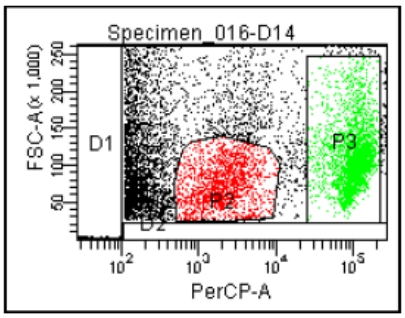


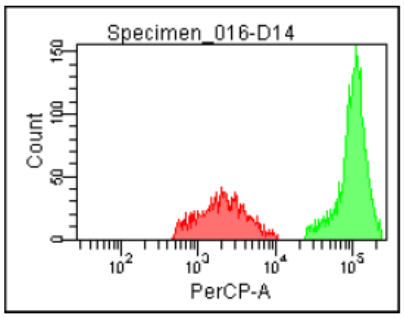
Tube: D2

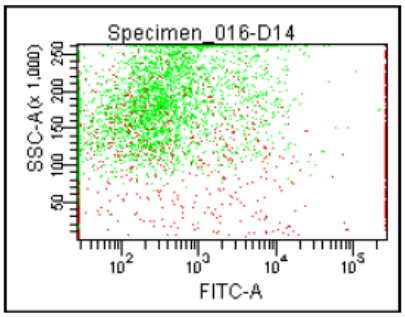
Population	#Events	%Parent	%Total
All Events	731,538	####	100.0
D1	700,198	95.7	95.7
NOT(P1)	31,340	4.3	4.3
P2	569	1.8	0.1
P3	5,770	18.4	0.8
D2	541	1.7	0.1

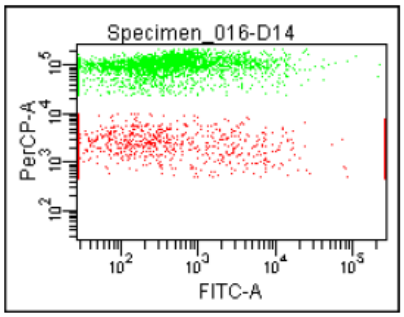






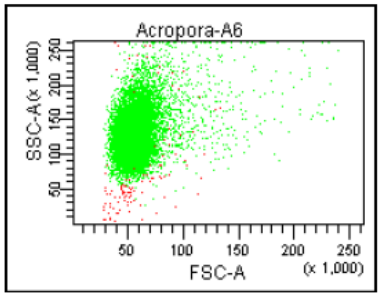


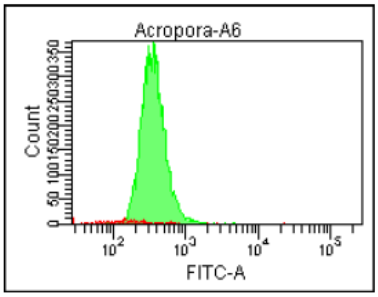


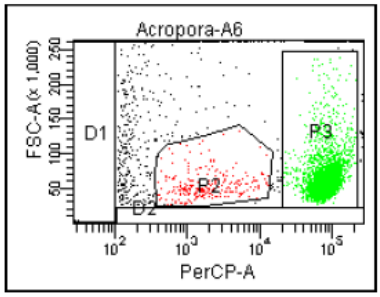


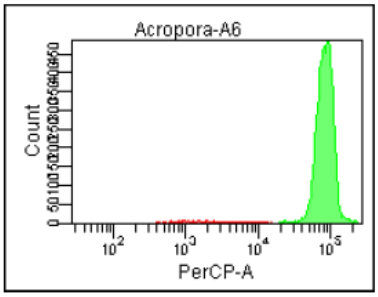
Tube: D14

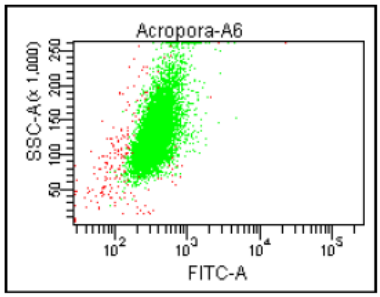
Population	#Events	%Parent	%Total
All Events	822,055	####	100.0
D1	805,265	98.0	98.0
NOT(P1)	16,790	2.0	2.0
P2	1,619	9.6	0.2
P3	2,944	17.5	0.4
D2	1,290	7.7	0.2

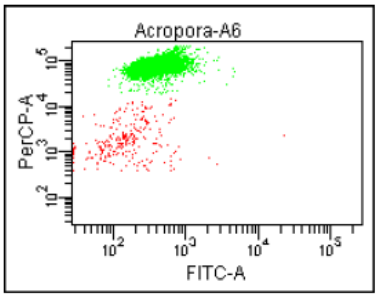






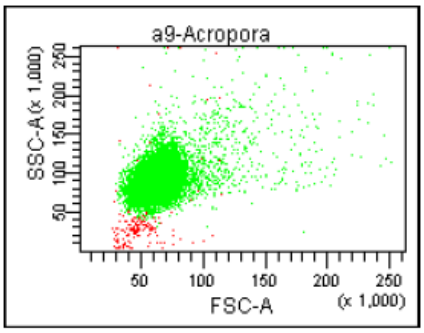


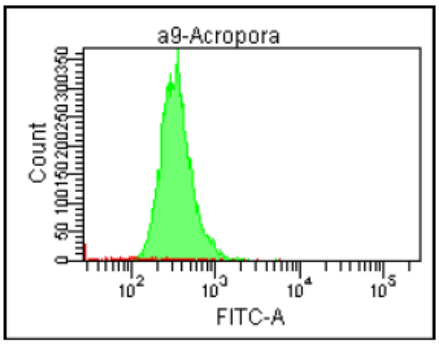


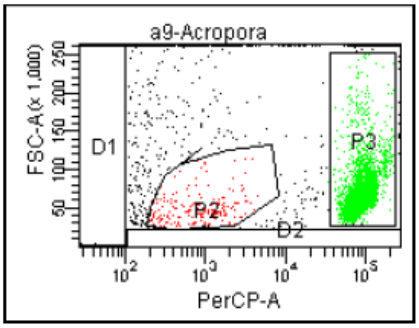


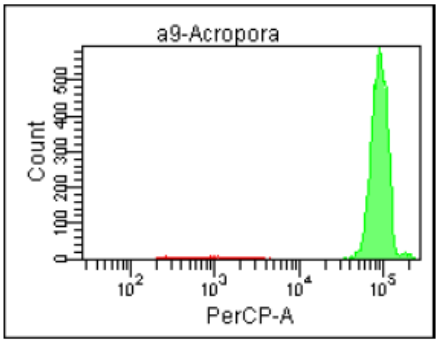
Tube: A6

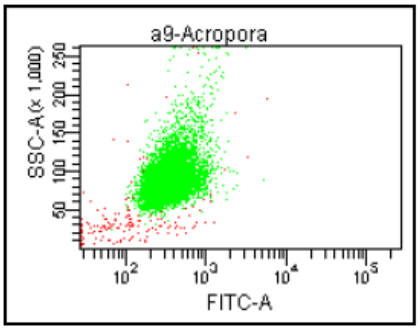
Population	#Events	%Parent	%Total
All Events	117,947	####	100.0
D1	107,638	91.3	91.3
NOT(P1)	10,309	8.7	8.7
P2	257	2.5	0.2
P3	8,571	83.1	7.3
D2	146	1.4	0.1

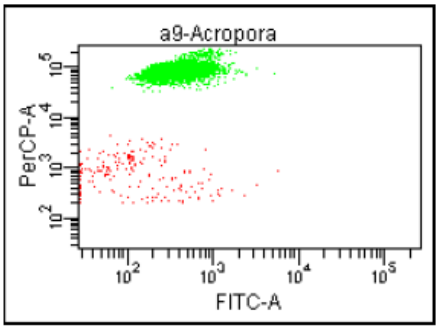






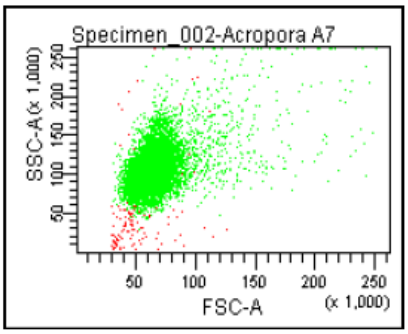


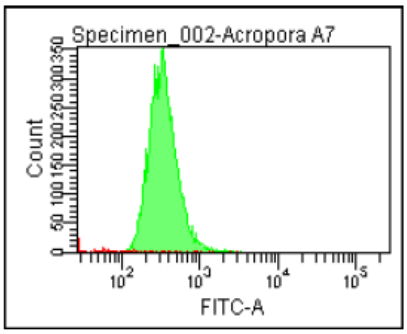


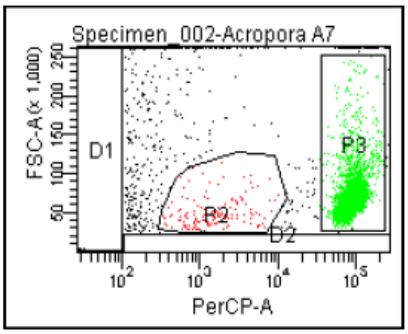


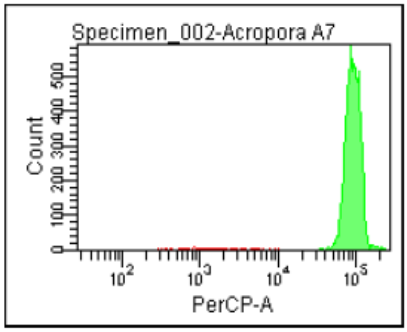
Tube: Acropora

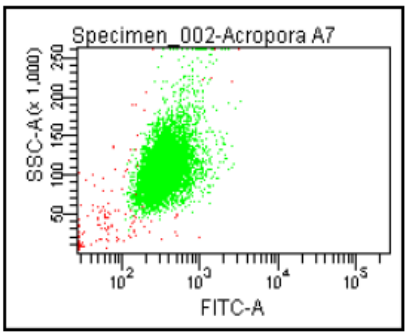
Population	#Events	%Parent	%Total
All Events	78,477	####	100.0
D1	68,002	86.7	86.7
NOT(P1)	10,475	13.3	13.3
P2	187	1.8	0.2
P3	8,709	83.1	11.1
D2	258	2.5	0.3

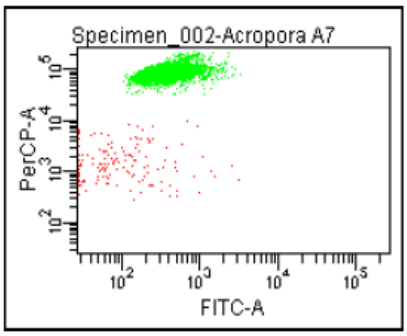












Tube: Acropora A7

Population	#Events	%Parent	%Total
All Events	78,832	####	100.0
D1	68,811	87.3	87.3
NOT(P1)	10,021	12.7	12.7
P2	155	1.5	0.2
P3	8,490	84.7	10.8
D2	258	2.6	0.3