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Distinctive physiology (growth and photosynthetic efficiency) of Qatari corals under variable dynamic factors, an experimental approach

A Thesis in
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By

Nayla Mohammed Al-Neama

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THESIS APROVAL

The thesis of Miss **Nayla Al-Neama** was reviewed and approved by the following:

We, the committee members listed below accept and approve the Thesis/Dissertation of the student named above. To the best of this committee's knowledge, the Thesis/Dissertation conforms the requirements of Qatar University, and we endorse this Thesis/Dissertation for examination.

Name ____Dr. Radhouane Ben Hamadou_____

Signature _____ Date_____

Name _____Dr. Suhur Saeed_____

Signature _____ Date_____

Name ____Dr. Mohammad A. S. Al-Ghouti_____

Signature _____ Date_____

Name _____Dr. Mohammed Abu-Dieyeh_____

Signature _____ Date_____

ABSTRACT

The Arabian Gulf is well known as being probably the most extreme environment in which zooxanthellate coral reef communities occur. A combination of both extremely high and low temperatures as well as high salinities, combined with shallow profile of the coastal waters (high luminosity) imply high stress for coral physiology that may explain much of the coral bleaching events observed during the last decade. We proposed to investigate corals *Symbiodinium* response to different levels of those three elements. Among the different available methods; fluorescence technique (Imaging Pulse Amplitude Modulation - PAM) and buoyant weight were used to assess photosynthetic activity and health stress. *Porites sp.* samples were collected from two Qatari marine areas and exposed to high levels of the three essential elements in controlled laboratory conditions. Our results suggested that Imaging-PAM parameters (Maximum Quantum Yield Fv/Fm, Electronic Transfer Rate ETR, and Non-Photochemical Quenching NPQ) and growth gain percentage provided useful routine tools to detect stress situation in hospite *Symbiodinium* in *Porites sp.* Moreover, the high resolution images derived from PAM were able to capture the stress before it become visible to observer eyes. Elevated levels of temperature (38 °C) and salinity (50 psu) negatively influenced corals health and caused death events. Coral bleaching started after the sixth day of exposure suggesting that coral are affected only when extreme conditions persists for more than one week. With regards to raised light intensity, no stress was detected; in contrary growth and photosynthetic efficiency increased.

Keywords: Corals, *Porites, Symbiodinium* Arabian Gulf, Imaging- PAM

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LIST OF ABBREVIATION

Img-PAM	Imaging Pulse Amplitude Modulation Flurometer
PAM	Pulse Amplitude Modulation Flurometer
Y(II)	Maximum Quantum Yield of Photosystem II
Fm	Maximum Fluorescence
Fv	Variable Fluorescence. Calculated as Fv= Fm-F_0
ETR	Electronic Transfer Rate
NPQ	Non-Photochemical Quenching
PAR	Pulse Amplitude Rate

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CHAPTER I: Introduction

1.1 Arabian Gulf Ecosystem

The Arabian Gulf is an extension of the Indian Ocean; where it is located from Iran (Northeast) to Arabian Peninsula (Southwest) (Figure 1). The Gulf as a whole is dominated by soft substrate ecosystems. Elevation and depth contours between Kuwait and the UAE are generally extremely gradual; leading to a highly sedimentary environment, encouraging growth of seagrasses and algal beds, of particular significance several so-called critical marine habitats (Ray, 1976). Ecological attributes of these include: high biological productivity; provision of nutrients, nesting areas; areas particularly rich in species (e.g. coral reefs); and areas important for sustaining populations of species (e.g. seagrasses, shrimp, pearl oysters, dugong and green turtles) (NOAA, 2007). Corals are one of the most important marine habitats in the Gulf area; their existence is very vital because they act as engineer species providing suitable habitat that will support and nourish multiple marine species (Bledzki, 2010). According to the Environment Agency in Abu-Dhabi (2012) the number of species that are associated with the reef in Qatar is up to 48% out of the total numbers presents in the Arabian Gulf. Besides that, corals are considered as bio-indicators of the Gulf health, reflecting the environmental conditions of the Gulf (Valavi et al., 2010)

Figure 1. A map of the Arabian Gulf (Google

1.2 Decline of the Arabian Gulf Corals

Limitations to coral growth come from extreme environmental conditions, and possibly from barriers to recruitment (Sheppard et al., 1992). Communities exist in harsh environment with respect to salinities, sea temperatures and extreme low tides (Coles 1990; Sheppard and Sheppard 1991; Coles and Fadlallah 1991; Sheppard et al., 1992). Important environmental conditions which may limit coral diversity are high and low water temperatures. Seasonal temperature changes, however, appear to be as important as absolute temperature values. In shallow areas, seasonal temperature fluctuations of up to 35°C occur, and in such areas the numbers of corals which can survive are fewer than 20 species (Sheppard et al., 1992). The Arabian Gulf is one of the few areas in the world where corals occur in region with elevated salinity (Coles and Jokiel, 1992). The Arabian Gulf salinity average is around 42 psu in open water (John et al., 1990). Salinity further increases to 70 psu in bays in the Gulf of Salwah (Coles and McCain, 1990; John, et al., 1990).

Furthermore, land reclamation projects, elevated sea temperatures and sea pollution have been cited as major causes of coral mortality in Gulf waters (Sheppard et al. 2000) Lately, there has been a dramatic decline in the coral population of the Gulf area due to both anthropogenic and non–anthropogenic pressures and the results reflected on number of species that relay on the corals for their continued existence. While quantitative information exists for coral communities in the Arabian Gulf (Downing 1985; Sheppard and Salm1988; Coles and Fadlallah 1991; Fadlallah et al., 1993; Vogt 1996), little work has been done to understand corals physiology and health under a changing environment. Since human populations are inhabiting tropical coastal areas derive great value from coral reefs, the degradation of these ecosystems, as a result of coral bleaching and its associated impacts, is of considerable social as well as biological concern. Efforts to forecast and monitor bleaching, involving both remote sensed observations and

coupled ocean–atmosphere climate models, are also underway. In addition to these efforts, attempts to minimize and mitigate bleaching impacts on reefs are immediately required. In-order to close the gap between field and laboratory observations, laboratory experiments under controlled conditions are required.

1.3 Research Focus

The main purpose of this study was to detect biological responses in corals to different levels of stress stimuli: salinity, light intensity and temperature using non-conventional technology (Pulse Amplitude Modulation Fluorometry). The advantages of using this technique are: laboratory based, rapid, non-invasive and gives high resolution imagery that shows the photosynthetic activity of the corals.

CHAPTER II: Background and Literature Review

2.1. Coral Physiology

Corals are marine invertebrates of class of Anthozoa of phylum Cnidaria (Gates, 1999). Stony corals are colonial reef-building organisms, building an external calcium skeleton (Merks, 2002). Corals can exist as individual polyps, or in communities that contain hundreds to thousands of polyps (Barnes, 1987; Lalli and Parsons, 1995). An individual coral animal is called a polyp which is a soft-bodied and lives inside a hard cup-shaped, skeleton, made of calcium carbonate called a corallite (Tomascik et al., 1996). Polyps are multi-cellular and their cells exhibit specialization to perform various functions including gastro vascular cavity (simple stomach) that opens only on one end, and a ring of tentacles which surround central mouth opening; the tentacles are used for: defense, capture small animals (e.g. zooplankton) as food and clear out the debris (NOAA, 2007). Tentacles contain special cells called nematocysts; they are capable to deliver toxins to capture their prey (Barnes, 1987). Another function of the polyps is that they take calcium ions from adjacent water and express an alteration to finally secrete calcium carbonate structures (NOAA, 2007). Individual heads grow by asexual reproduction of polyps or sexually by spawning where polyps of the same species release gametes simultaneously over a period of one to several nights around a full moon (Sumich, 1996; Veron, 2000). The group of corals will consolidate the coral reef, the reefs are one of the oldest and most diverse ecosystems on earth; they offer the nurseries and feeding foundation for fish and invertebrates and natural storm barriers for coastlines (Hagedorn et al., 2010). Also, scleractinian corals are considered to be one of the major benthic primary producers in coral reefs because they have endosymbiotic photosynthetic algae in the host animal tissue (Tanaka et al., 2014). Corals are covered by unicellular dinoflagellates algae in the genus *Symbiodinium* (often referred to as symbionts,

endosymbionts or zooxanthellae) that produce energy-rich compounds in exchange for the carbon substrates needed for photosynthesis (Hagedorn et al., 2010). Zooxanthellae and corals have mutualistic relationship; coral provides the algae with living habitat and compounds they need for photosynthesis, in return, the algae convey oxygen, give corals unique color and supply the coral with glucose, glycerol, and amino acids (NOAA, 2007). The corals utilize these products to construct proteins, fats, and carbohydrates (Barnes, 1987; Lalli and Parsons, 1995; Levinton, 1995; Sumich, 1996 and Barnes and Hughes, 1999) In fact, almost 90 percent of the organic material that processed by zooxanthellae is transmitted to the multitude coral tissue (Sumich, 1996). Thus, they motivate coral reef growth and productivity (Barnes, 1987; Levinton, 1995).

2.2 Bleaching

Several environmental factors such as changes in light levels and seawater temperature can trigger the loss of zooxanthellae from the host (Brown, 1997; Douglas, 2003 and Baker et al., 2008). When the visual appearance of the coral becomes dominated by the white color of the skeleton showing through the tissue, the phenomenon is known as "bleaching". Bleaching due to thermal stress is considered to be induced by the algal production of reactive oxygen species (ROS), particularly H_2O_2 (Smith et al., 2005; Lesser, 2006; Suggett et al., 2008 and Tchernov et al., 2011). Although the animal host might survive and recover, mass mortality is frequently observed among bleached corals (Glynn, 1996; Wilkinson, 1998 and Baker et al., 2008). Although bleaching severity and recovery have been varied across all spatial scales, some reefs have experienced relatively rapid recovery from severe bleaching impacts (Baker et al., 2008). The algal cell density in corals is approximately on the order of millions per square centimeter of the coral surface area and is affected by environmental changes such as seawater temperature

(Fagoonee et al., 1999) and light intensity (Masuda et al., 1993). Occasionally when corals express physical stresses the polyps expel their zooxanthellaes and presenting white appearance (Krediet, 2013). This is commonly described as "coral bleaching" (Lalli and Parsons, 1995; Barnes and Hughes, 1999) which is characterized by reductions in zooxanthellae densities; leading to photosynthetic impairment of photosynthetic function and changes in mitotic index (Jones 1997; Hill et al. 2004). Over the last 17 years recurrent local, regional and global bleaching events resulted in significant coral mortality. This response of corals usually observed in more extreme environmental challenge and received a considerable amount of attention (Brown, 1997). Corals encounter multiple natural threats; weather correlated like strong waves that can break coral reefs into fragments (Jones and Endean, 1976; Barnes and Hughes, 1999) or increase in seawater temperature leading to damage to photosynthetic and mitochondrial membranes (Jones, 1997; Weis, 2008 and Higuchi et al., 2010). In addition, increased sea surface temperatures, decreased sea level and increased salinity, collectively these environmental conditions can have distressing results on a coral's physiology (Forrester, 1997). Other threats could be infections caused by bacteria, fungi and viruses (Santavy and Peters, 1997) that increase on the total suspended solids in the water column (Flores et al., 2012) and predation on the soft polyp's tissue by fishes, worms, crabs and snails (Jones and Endean, 1976). Coral reefs may recover from natural occurrences or anthropogenic induced stressful conditions however; if corals are subjected to frequent or long lasting pressures including those forced by people, the physiological stress may exceed their level of tolerance and cannot recover their healthy conditions, and they will die (NOAA, 2007). Anthropogenic activities; or humane-induced activities considered one of the main threats to the coral reefs such as: pollution, overfishing and

removing corals from their natural environment (e.g. to aquarium market) and others activities are recognized as main pressures of coral reef loss around the world (Bryant et al., 1998).

2.3 Status of the Corals in the Arabian Gulf

The Arabian Gulf is a semi-enclosed, very shallow sea (average depth 35m); with limited water exchanges and extreme evaporation rates which creates temperature ranges of (10 to 40°C) and salinity of (28 to 60 psu) and the combination of these factors causes extreme conditions for coral growth (Wilson et al., 2002). Its photic zone mostly extends to only 6–15 m (Siebold, 1973). Many of the 'coral reefs' described for the Gulf are areas of hard substratum which are not actively accreting but are modern veneers of living coral on much older limestone domes or recently formed diagenetic hardgrounds, many of which are visually indistinguishable from true reefs (Shinn, 1969). Communities range in composition from large monospecific stands, mostly of *Acropora* and *Porites spp.* to more diverse assemblages composed of massive poritids and faviids (Vogt, 1996). Along the Arabian Peninsula, coral assemblages show best development offshore, but there are important fringing systems too (in particular Abu Dhabi, Qatar and Saudi Arabia) (Sheppard et al., 2009). In the Arabian Gulf, reefs are known to support < 100 species of stony corals and ca. 600 spp. of reef-associated fishes. The average live coral cover was 33 % during the 1992 and 1994 surveys and showed slightly decreased to 31% in 1999 (DeVantier et al., 2000) These reefs survived the effects of the Gulf War oil spills, but were affected by high sea surface temperatures, which exceeded 34 ºC in summer 1998 (Vogt, 1996; Vogt and Al Shaikh, 2000). Another survey that was done in 2000 showed that coral reefs were in healthy conditions from different threats (Krupp and Almarri, 2000) Qatar has seen striking coral decline, a condition typical of the Gulf generally (Sheppard et al., 2009) and notable high coral mortality was also reported in the southeastern Arabian Gulf (United Arab Emirates and Qatar)

in1996 (mortality >90%), which after nearly a decade had only recovered in a small area (Burt et al., 2008). Conducted surveys in (2007–2008) found only 20 species of hermatypic coral, and only five species belong to three genera at the offshore island of Halul (SCENR, 2007; Qatar Ministry of Environment).

2.4 Pulse Amplitude Modulation Fluorometer

Zooxanthellae supply corals with the essential photosynthetic products; thus the symbiont photosynthesis is critical process to the survival of corals (Hill et al., 2004). Different factors control photosynthetic efficiency of corals (Gladfelter et al., 1989, Helmuth et al., 1997 and Jokiel et al., 1997), including different genetic strains of zooxanthellae that display different photosynthetic capacities (Rowan and Knowlton, 1995). Microhabitat variations in light, water flow, and gas exchange appear to strongly influence the photosynthetic responses of the zooxanthellae (Ku¨hl, 1995; de Beer et al., 2000). Research done by Roff showed that even if there are no significant differences observed in *Symbiodinium* biomass, changes in the photosynthetic efficiency of symbionts may occur long before visible signs of pigment loss or lesion development in the coral host (Roff et al., 2008). Chlorophyll fluorescence measurement is a very powerful and useful tool for monitoring the physiology of symbiotic dinoflagellates within scleractinians (Fitt et al., 2001). Recently, a tool was developed called Imaging-PAMchl fluorometer to study the induction and quenching of chlorophyll fluorescence in physiological studies (Schreiber, 2004). This submersible or laboratory based instrumentation technique has become both an efficient and relatively inexpensive method of collecting data in vivo; allowing for complete non-invasive measurements to be made (Beer et al., 1998; Ralph et al., 1999). It applies pulse amplitude measuring light (using blue light-emitting diodes [LEDs]) to map the chlorophyll a fluorescence yield with a spatial resolution of <0.5mm (Hill et al., 2004). Also, it

can perform all standard routines of saturation pulse quenching analysis, such as determination of Fv/Fm (Maximum PSII quantum yield), as well as measurements of fluorescence induction and rapid light curves (Ralph et al., 2002). Fv/Fm is a robust indicator of the degree of potential photosynthetic competence, nutrient stress and photoinhibition of phytoplankton, and the measurement of Fv/Fm in phytoplankton has contributed greatly to the developing field of the study of aquatic photosynthesis and ecosystems (Bergmann et al., 2002; Cavender-Bares and Bazzaz, 2004). In addition, the Imaging-PAM offers a special routine in which images of PAR absorptivity are obtained (Hill et al., 2004). The advantages of the PAM fluorescence technique include its ability to capture a great deal of information related to the photosynthesis of phytoplankton in a convenient, rapid and non-invasive manner, and to assure high spatiotemporal resolution measurements in the study of aquatic photosynthesis (Goto et al., 2008)

CHAPTER III: APPROACH / METHODOLOGY

3.1 Research Strategy

Little is known about corals maintenance under controlled laboratory conditions, how they would perform under artificial conditions including stress stimuli, and how can we capture their health condition (physiology). Therefore, in this research study series of laboratory experiments were conducted and used the data obtained to answer all of these questions. From preliminary studies we found that there are three main parameters that are important for corals growth. These parameters include salinity, light intensity and temperature. To investigate this further, studies were designed and carried out in a short term for two weeks and used a custom designed (microcosm) for each of the three parameters. In brief the experimental design composed of testing one variable while keeping others constant. For example, my plan included two levels of salinity (40 PSU and 50 PSU), three different light intensities (6900 Lux; 8300; and 12000 Lux) and three set of temperatures (24; 30 and 38 $^{\circ}$C). To avoid pseudo-replication and properly increase the precision of our estimates, replication of the experimental units was considered while applying replication within units (several coral nubbins per experimental unit).

PAM and buoyant weight were used to evaluate photosynthetic process and potential impact on corals health and growth. Water quality and nutrients were monitored for the duration of the experiment.

3.2 Materials

Plexiglass plastics were used to make the microcosms. Media was made from commercially available artificial seawater with formulations that is designed to closely mimic natural seawater chemistry. Testing kits used for the water quality parameters were purchased from (JBL©, Germany); pH measurement were carried out using VWR® instrument (sympHony™ Handheld

Meters, UK) Light was provided via LED aquarium light with three light spectra (white, yellow and royal blue). Light measurements were recorded manually (WALZ-ULM-500, Germany) and using automated sensor (HOBO Pendant Temperature/Light Data Logger, USA). Corals were fed once a week with brine shrimps. Aerations were provided through aquarium air pumps connected to air stones in each semi-microcosm. Water temperature was maintained using aquarium heaters; temperature was recorded manually VWR® and using HOBO sensors. (WALZ IMAGING-PAM *M-Series*, Germany) was utilized to record measurements of Maximum Quantum Yield Fv/Fm, Electronic Transfer Rate ETR, and Non-Photochemical Quenching NPQ.

3.3 Corals Collection

Corals samples were collected from different location in Qatar (Umm Al-Arshan/ N 26°30'49.8" E 51°17'58.4" and Fuwayirt/ N 26°01'42.3" E 51°23'09.5") (Figure 2). The collection was handled by professional third party corals specialist divers. Pieces of corals "nubbins" was cut by hammer and bolt from mother's colonies and added to plastic containers that contain sea water from original area of collections. Small containers were kept in bigger container and temperature was maintained as much similar as possible to the temperature of original location. Upon receiving corals nubbins, physical examination was performed to determine the health of the corals (stressed corals are usually pale and tentacles are not shown).

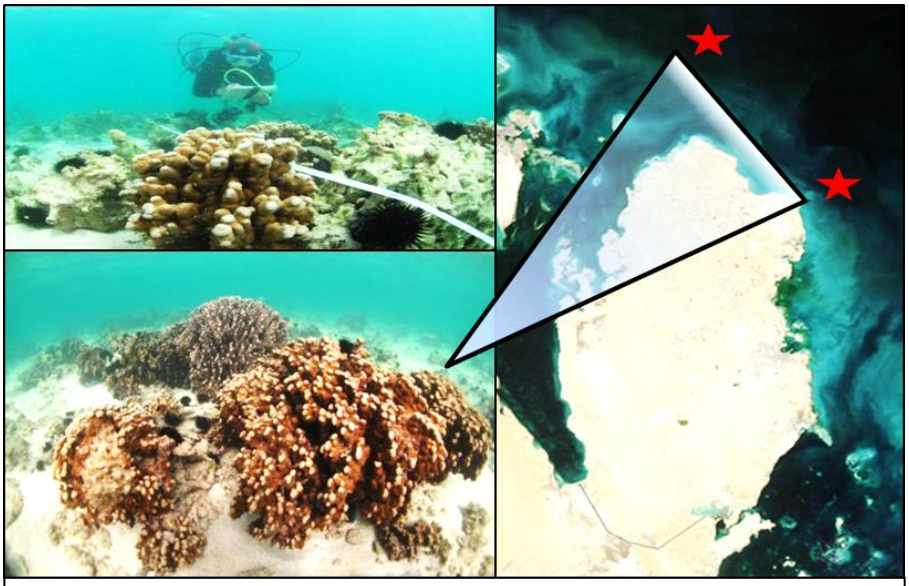


Figure 2. Qatar Map with collection areas (red stars) and divers to collect samples from mother colony of *Porites*

3.3.1 Acclimatization

Acclimatization process started immediately after reception at laboratory by adding collected corals gradually into pre-acclimatized laboratory aquarium (process that might take 3-5 hours). All tanks were kept in the same conditions; light of 6900 Lux, salinity 40 psu and water temperature 24°C. Then tank water was added gradually (artificial seawater) to the collected water and wait for an hour with close observation of the status of the corals; followed by a full transition of the corals into the microcosm and monitor corals for 48 hours for any stress symptoms. Microcosm aquarium closely simulating the functions of the shallow water, were run on a closed system. This system was properly equipped with filtration unit consisting of protein skimmer to remove dissolved organic compounds, water pumps to circulate water and mimic flow current and biological and mechanical filtration equipment to remove debris and allowing beneficial microorganism to grow and help to keep high water quality (Figure 3). Additionally the aquarium is provided with aquatic LED light that mimics sun light intensity and diurnal cycle to allow photosynthetic process of the corals to occur in natural conditions.

Water parameters in the aquarium were maintained as similar as possible to the ones found in their natural habitat. Continuous monitoring for the corals was carried out for at least one to two weeks before the start of the experiment. Initial PAM records were done before start of experiments to assess corals health and identify photosynthetic process at t_0 (before starting experiment). A wide range of water quality parameters were monitored every week, including; ammonia, nitrate, nitrite, phosphate and calcium. Temperature, salinity and pH were also recorded every day. Corals were fed on livestock/ frozen baby brine shrimps once a week as a supplementary food.

Figure 3. The setup of laboratory microcosms aquariums with controlled light, temperature and salinity

3.3.2 Corals Conditioning

To start experiment, it is easier to have the nubbins in small size (thumb size) because they will be easier to handle, and proceed with weighting and imaging-PAM records. From one single big nubbin (corresponding to the size of a tennis ball) we can end up by having 5 nubbins. Corals were divided using a metal sterile cutter. Once corals were acclimatized for 48 hours, the basing process started. It is necessary to attach the corals to a base so they can stand naturally and grow horizontally and vertically. Plastic rulers were cut into small pieces according to our need; corals were attached using plexiglass glue (adhesive glue) which is a well-known inert material so it

will not affect the corals as I found that corals showed a satisfactory growth on it (Figure 4). This substratum revealed to be easy to use, widely available and an affordable solution. As a best practice, when attaching nubbin to these substrate, it is recommended to press it once in steady phase with little pressure on the glue; thereby it will attach immediately; allowing for natural growth and spread, while if you press the nubbin for multiple time in different directions; this will affect growth of the edges and the nubbin will be covered by the glue.

Figure 4. Process of cutting corals and attach them to artificial substratum (plastic rulers) using a plexiglass glue

3.4 Experimental Design

Experiments were designed in a way to have separated compartments and could be fitted inside the incubation aquarium so there was no need to change the initial light/ temperature settings of the corals; hereby, we built several microcosms with a singular volume of around 25-30 L. It can contain adequately around 9 nubbins of corals with putting automated data loggers at the level of the coral nubbins to monitor temperature and light intensity. Additional advantage is that this

setting will allow us to run independent replicates since we are able to have multiple microcosms running at the same time, thus mixing the use of the available space into the aquariums.

3.4.1 Salinity Experiment

Six microcosms were set-up in the main aquarium (Figure 5), three control replicates (40 psu) and three treatment replicates (50 psu). Each microcosm contained five nubbins of *Porites* (total samples # 30). Microcosms were distributed in a way to achieve equal distribution of light around all of them (Figure 6). Water flow was maintained in the containing tank in a way to maintain the temperature at 24 ±1°C. Salinity was increased in the treatment microcosm every 3-days by 3 psu until desired salinity was reached (50 psu). The experiment was carried out for duration of 12-days at constant salinity. PAM measurements were taken every 3 days until the end of the experiment. Weight of individual corals was measured at the beginning and at the end of the experiment. Water quality was done every day. Seawater inside the microcosms was renewed manually every day by removing 2 L and adding 2 L of freshly prepared artificial seawater. Aeration supplies were introduced to all the microcosms using aquarium diffuse stones

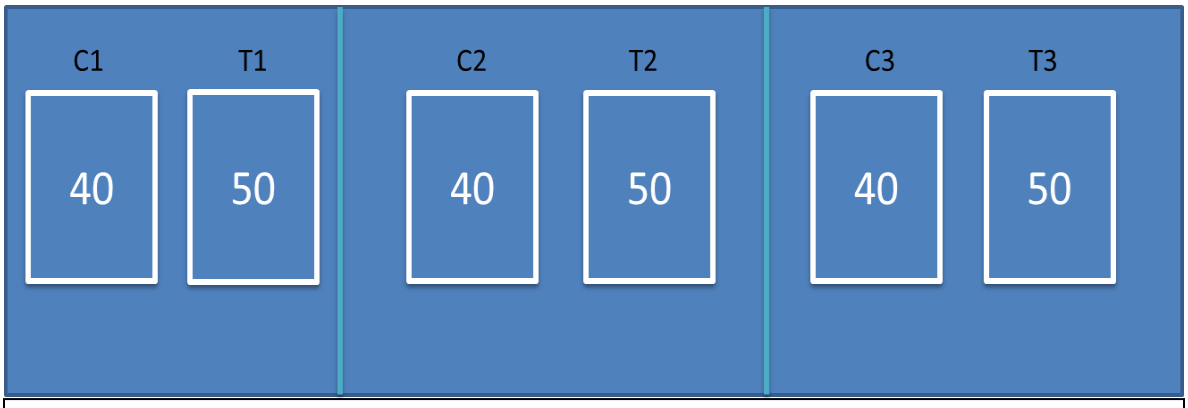


Figure 5. Diagram illustrating the set-up of the salinity experiment

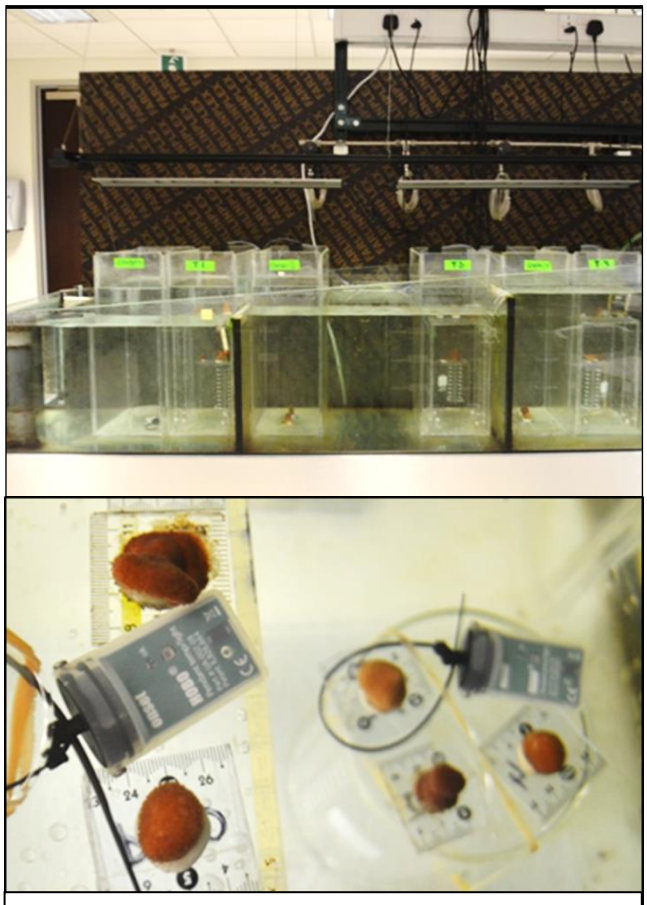


Figure 6. Salinity setting-light off at night and HOBO data loggers

3.4.2 Light intensity Experiment

Six microcosms were set-up in the main aquarium (Figure 7) with three light intensities; three controls (6900 Lux) and three treatments that were divided into 2 compartments with two different elevations (Elevation1: 8300Lux; Elevation 2: 12000Lux). The light intensities were set by placing the coral nubbins at different heights (Figure 8). Each replicate contains three nubbins (total number # 27 nubbins). Samples were introduced to different light intensity and kept for 3 days to be acclimated. Water flow was maintained in the containing tank to keep at a constant temperature 24±1°C and salinity (40±2 psu). The experiment was conducted for 12 days and

PAM measurements were taken every three days until the end of the experiment. Weight was measured at the beginning and at the end of experiment Water quality parameters were monitored daily. Seawater was inside the microcosms were renewed manually every day by removing 2 L and adding 2 L of artificial seawater. Aeration supplies were introduced to all the microcosms using aquarium diffuse stones.

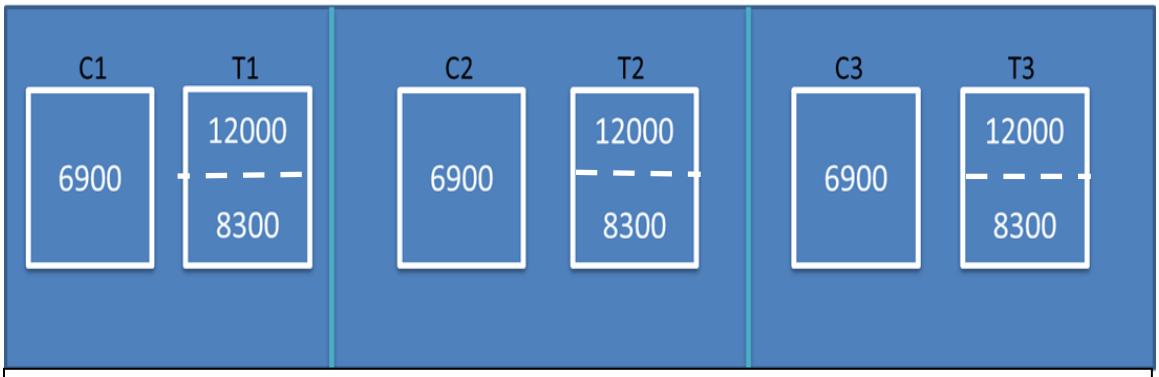


Figure 7. Diagram illustrating the set-up of the Light Intensity experiment

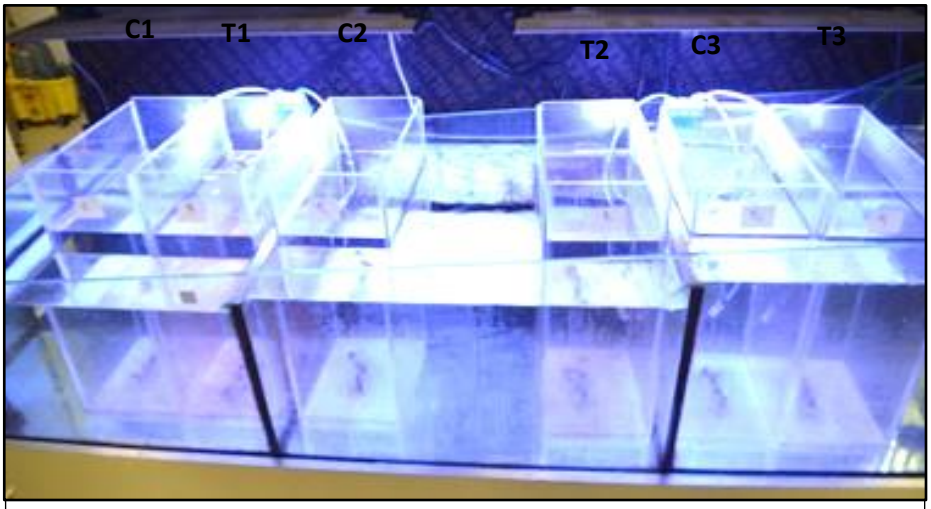


Figure 8. The actual laboratory set up of the Light

3.4.3 Temperature Experiment

The set up for the temperature experiment was slightly different to that of salinity and light experiments. The main aquarium was divided into three independent compartments with three microcosms in each compartment (Figure 9). First compartment contained three control replicates (24 ±1°C); second compartment had three treatment of (30 ±1°C); third compartment had three treatment of (38 ±1°C). Each microcosm had six nubbins (total sample # 54 nubbins). Temperature was gradually increased 3° C every 3-days by placing heaters into treatment microcosms and also outside within the aquariums. Experiment was run for 12 days at constant salinity (40 ±2 psu) and light intensity (6900 Lux). PAM measurements were taken in increments of three days until the end of the experiments. Weight was measured at the beginning and at the end of experiment. Water inside the microcosms was renewed daily by removing 2 L and adding 2 L of artificial seawater and water quality was monitored daily. Aeration supplies were introduced to all the microcosms for the duration of the study.

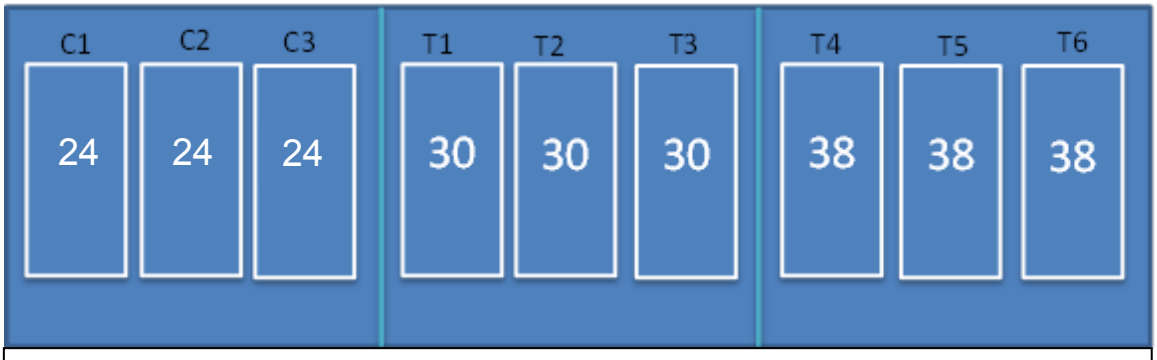


Figure 9. The set-up of the Temperature experiment

3.5 Analysis of Physiology Response Parameters

3.5.1 PAM

Chlorophyll a fluorescence of coral zooxanthellae was measured using a pulse-amplitude modulated fluorometer (PAM). A series of rapid light curve (RLC) measurements were performed on individual sample (nubbin) in crystalizing dish that has the same water from microcosm (Figure 10); the sample was then placed inside the imaging-PAM. After adjusting the position of nubbins, it was kept in the dark for about 5 to 30 minutes according to (Hill et al., 2004). RLC was obtained through the application of a series of light exposures with increasing irradiance (0, 1, 11, 21, 56, 111, 186, 231, 336, 461 and 531 µmol photos $.m^{-2}.s^{-1}$)

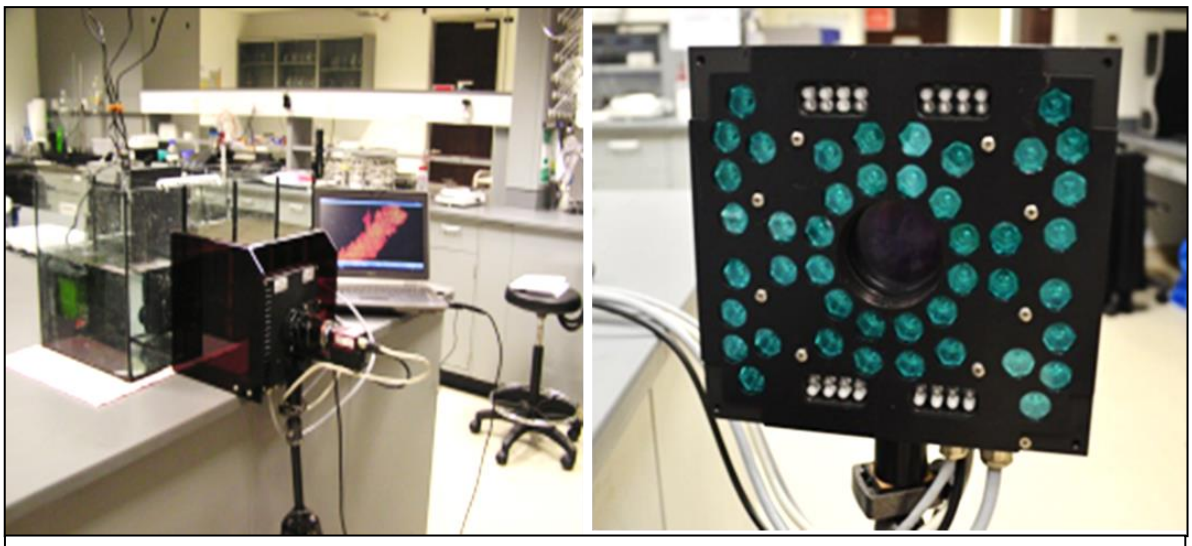


Figure 10. PAM Fluorometer technique

3.5.2 Weight Measurements

Buoyant weight technique is based on driving several simple equations (designated by letters) for volume and weight determinations. To obtain values for volume and weight the balance was allowed to auto-calibrate. Crystalizing dish that was filled with water was then placed inside the metal hanger and was allowed to settle down then auto-zeroed. A sample was taken in

crystalizing dish that was filled with the same water from the microcosm, nubbin was then transferred to the crystalizing dish inside the balance (Figure 11). After 5 min. the reading was settled and measurements were taken. Same steps were repeated for all other samples.

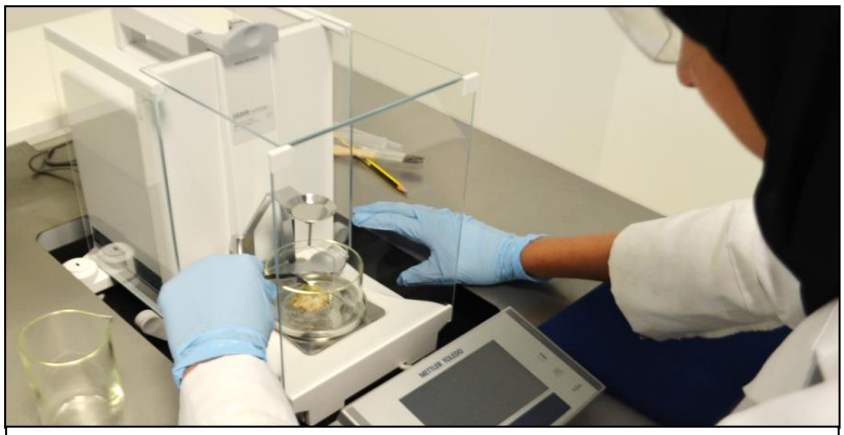


Figure 11. Recording weight measurements using buoyant weight

3.5.3 Physical observation

Physical observation and assessment were done through documentation of the appearance and color. Bleaching patterns were recorded at the onset of the bleaching process. Images were taken at the beginning and at the end of the experiments to capture any abnormal appearance using Nikon D-5100 series camera.

3.5.4 Water Quality Measurement

Small aliquots of seawater were removed from each microcosm to measure water quality. Salinity was measured with a refractometer and the pH with a laboratory grade pH probe and meter. Element such as calcium which is important in the formation of coral skeleton was measured using common aquarium test kits. Ammonia, nitrite, nitrate and phosphate are all waste or breakdown products that can accumulate over time and negatively impact coral health or affect experimental parameters. A number of commercially available kits were used to monitor their levels in the microcosms.

3.5.5 Statistical Analysis

Variations in the Y(II), ETR, NPQ and weight were tested using excel program, where significance was tested at the 0.05 level. To determine whether significant differences existed between control and treatments student's *t*-test was applied.

CHAPTER IV: RESULTS

4.1 Salinity Experiment

The effect of increased salinity was studied on Qatari *Porites* health and physiological condition. Salinity was increased gradually from 40 psu to 50 psu. The results obtained were then compared to that of control samples that were kept at 40 psu. The following are data obtained from the Imagining-PAM fluorometer that was used to analyze photosynthetic activity and images of chlorophyll fluorescence.

4.1.1 Maximum Quantum Yield (YII)

Response curves of Y(II) versus the number of days of the experiment are given in Fig. 12. *Porites* nubbins showed notable variation in the photosynthetic process absorptivity; where in the beginning of the experiment both control and experimental nubbins exhibited close values for Y(II) 0.48 and 0.58 respectively. However, monitoring the changes in Y(II) throughout the experiment it was noted that *Porites* nubbins responded differently to both salinity conditions. At salinity 40 psu the nubbins showed gradual increase whereas at 50 psu they showed significant decline ($p< 0.05$). The Y(II) values for the control were found to be 0.48 at the start of the experiment and 0.58 at the end of the experiment. On the other hand the Y(II) values for the treatment samples were found to be 0.58 at the beginning of the experiment and 0.28 at the end of the experiment.

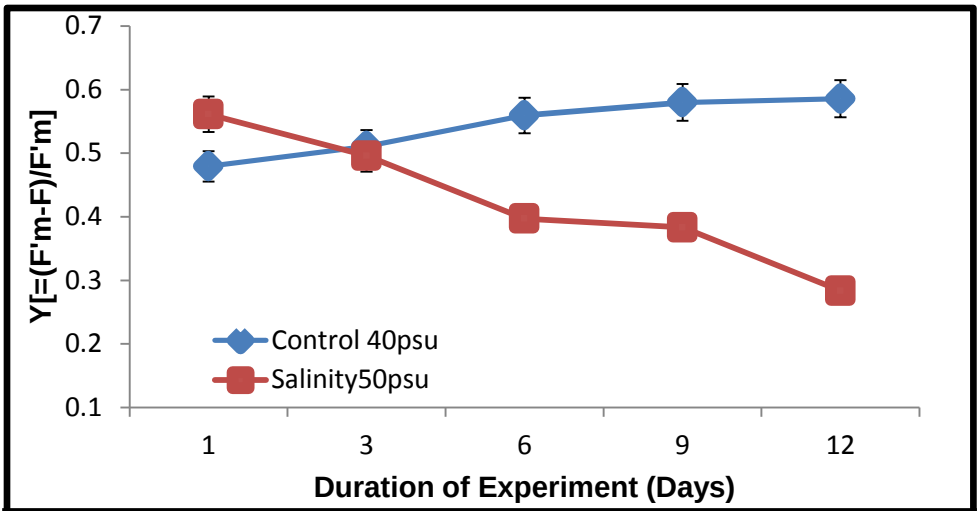
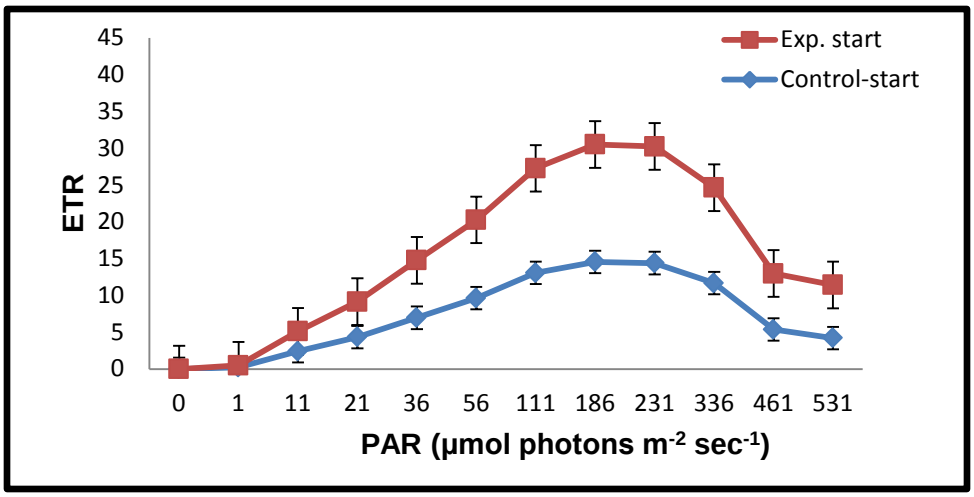
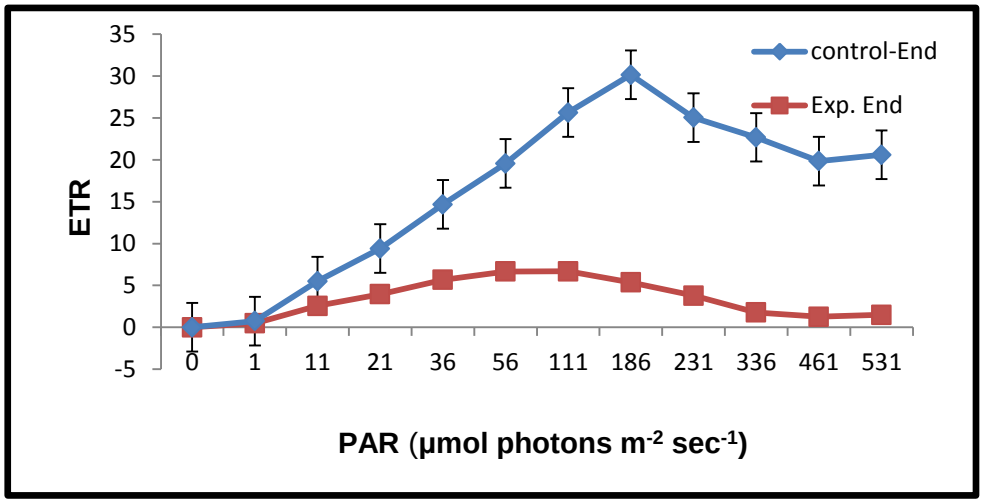


Figure 12. Response curves of Y(II) during the duration of the experiment. Two curves showing the responses of *Porites Symbiodinium* to two different salinity conditions (40 psu & 50 psu) P<0.05.

4.1.2 Rapid Light Curves

Measurements of ETR at the beginning and at the end of experiments for *Porites* nubbins are represented in (Figure 13). Samples were acclimated for 5-10 minutes in dark and then pulses of different light intensity (represented by PAR in x-axis) were emitted from PAM and recorded. For both control and treatment samples, first reading showed increasing in the ETR activity and then declined towards steady state. Nubbins absorbed light until they were fully saturated at around 200 PAR and then declined. Maximum ETR reading for control and treatment were 15 and 30 units respectively. The same measurements were repeated at the end of the experiment and showed similar pattern, ETR increased in both samples and reached their maximum around 200 PAR. However, both samples showed significant difference in their response($P < 0.05$), ETR value decreased dramatically in treatment samples while in the control samples as expected continued to increase and reached maximum at around 30. The NPQ value did not show differences between all samples and increased very slowly.





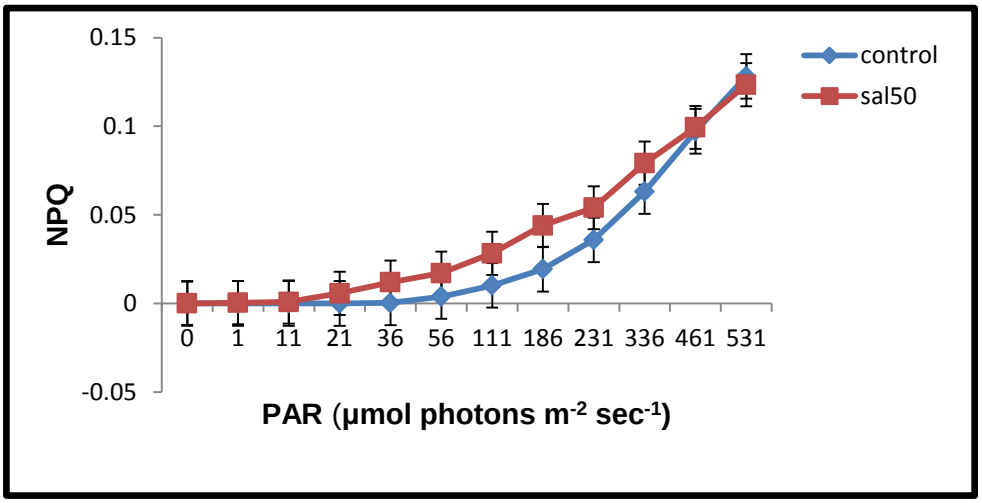


Figure 13. Recorded ETR and NPQ levels as function of irradiance (PAR) obtained by subjecting *Porites* corals to different salinity conditions.

4.1.3 PAM

Figure 14 shows images that were obtained from PAM after emitting different light intestines to the samples. High efficiency value will show purple and blue colors and lower values showing orange colors. Control maintained the photosynthetic efficiency with high levels of PAR until the end; whereas experimental unit could not use more light to be used for photosynthetic efficiency and shows black spots; indicating dead zone.

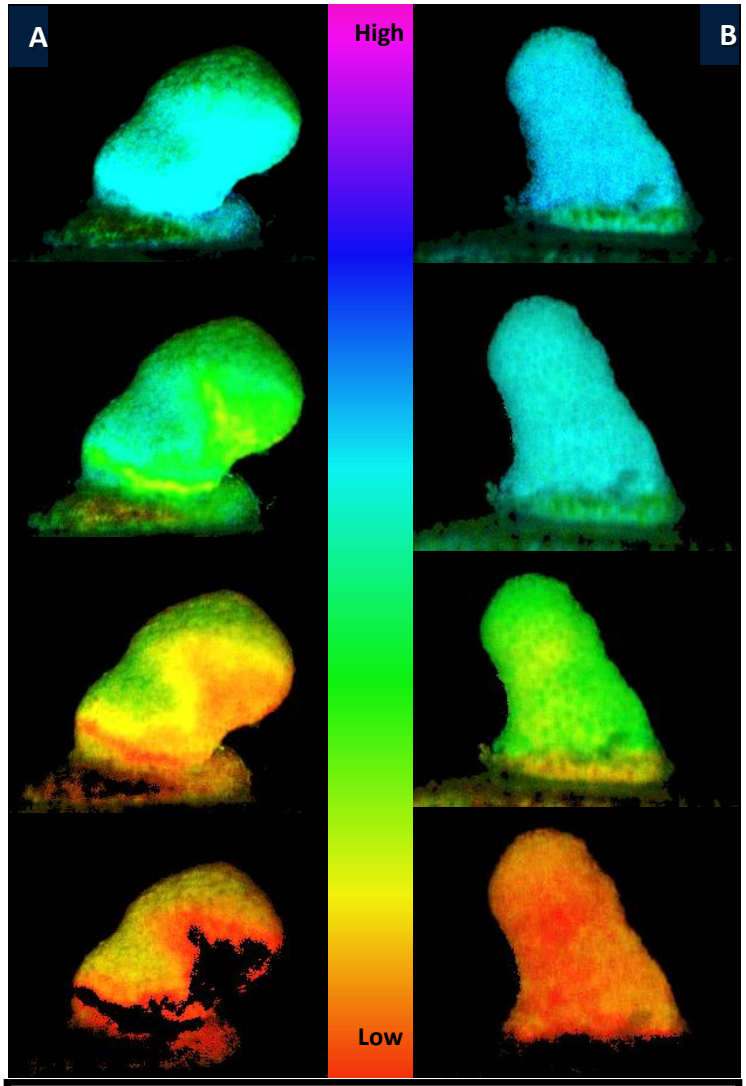


Figure 14. Images obtained from PAM of photosynthetic

4.1.4 Changes in Growth Rate

The growth of the nubbins was estimated from its buoyant weight in seawater and the differences were measured between the control samples and the treatment samples where salinity was increased to 50 psu. As shown in Fig. 15, the average weight gain for the control samples was 0.08 gm compared to decrease in average weight in the treatment samples by 0.03 gm.

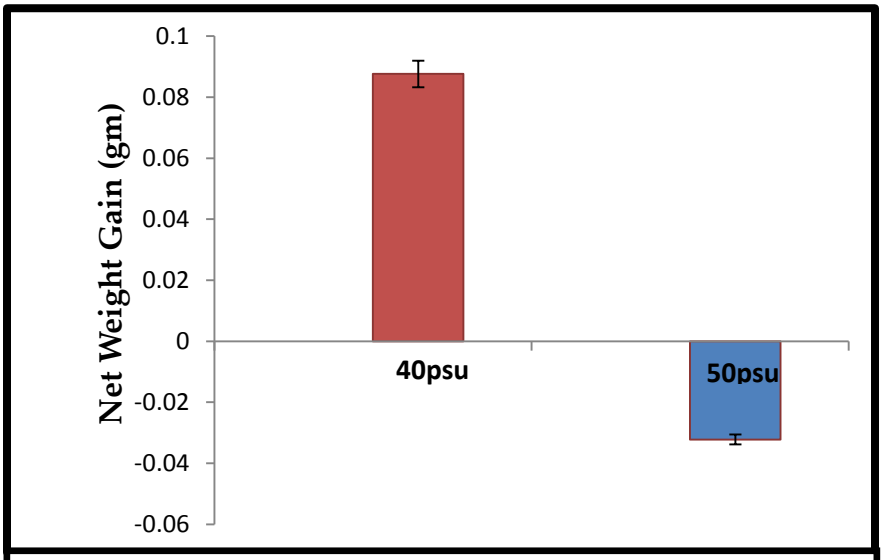


Figure 15. Net weight gain of *Porites* nubbins subjected to different salinity while keeping all other variables constant

4.1.5 Observation Monitoring

Pictures of the cultivated nubbins were taken regularly to assess the visible status of the corals in order to detect any sign of bleaching. Written records were maintained for each examination of the external features of the nubbins (Figure 16)

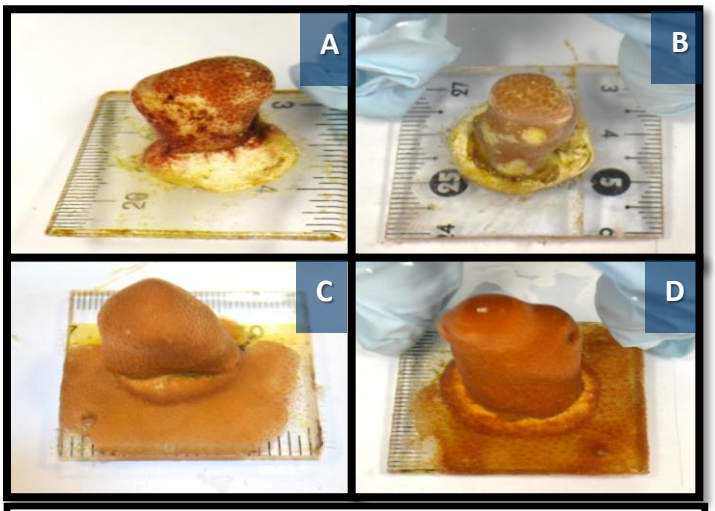


Figure 16. Pictures taken at the end of the experiment of control (C&D) and experimental units (A&B)

4.2 Light Intensity

Twenty seven nubbins were subjected to three different light intensities (Control: 6900 Lux; Treatment 1: 8300 Lux and Treatment 2: 12000 Lux) and the results obtained were then compared to that of control samples. The following are data obtained from the Imagining-PAM fluorometer that was used to analyze photosynthetic activity and images of chlorophyll fluorescence.

4.2.1 Maximum Quantum Yield (YII)

Changes in Y(II) were plotted against the number of days of the experiment (Fig. 17). There was a slight initial decline in Y(II) values for all samples after three days of exposure. On the sixth day of the experiment all nubbins exhibited the same behavior and showed an increased in Y (II) relative to the initial value. There was a significant difference between samples ($p > 0.05$).

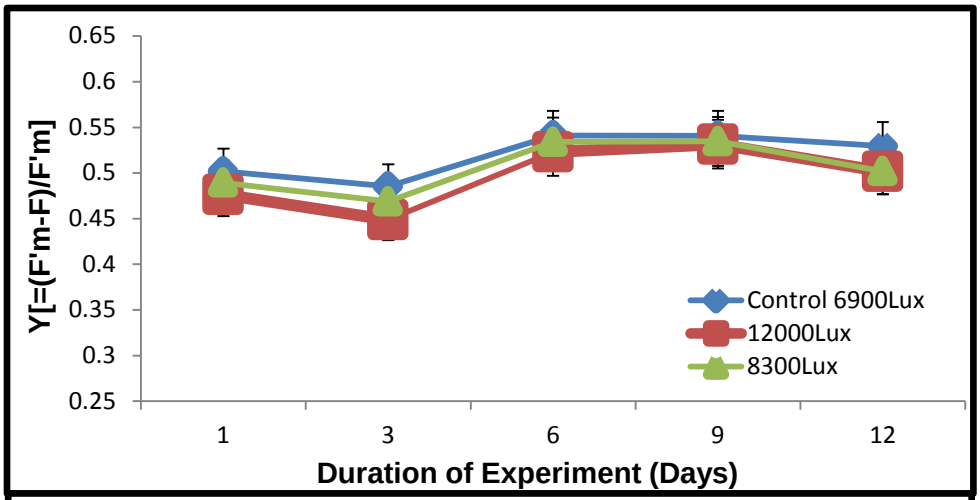
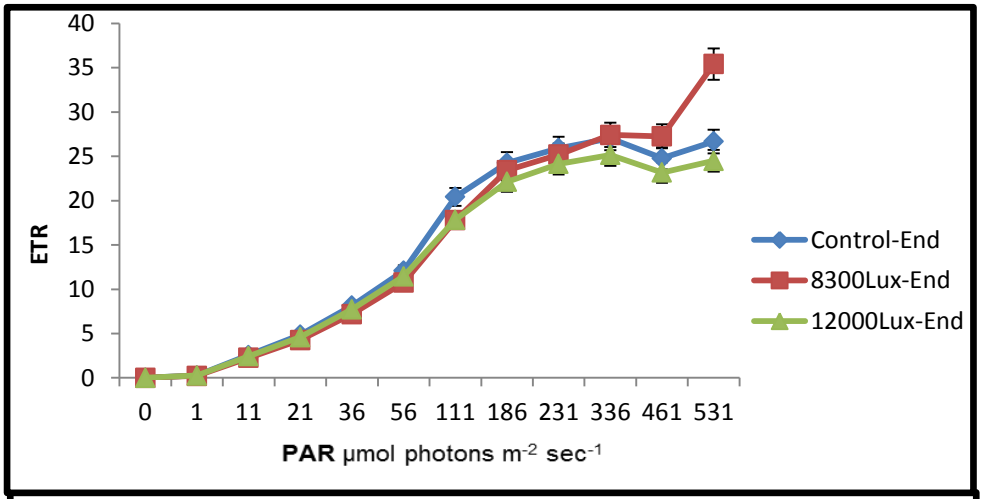


Figure 17. Response curves of Y(II) during the duration of the experiment. Three curves showing responses of *Porites* to three different light intensities (6900, 8300, 12000 Lux).

4.2.2 Rapid Light Curves

Measurements of ETR at the start of the experiment (Figure 18) showed an increase in ETR activity; reaching maximum of 18 at 230 PAR. Similarly, readings taken at the end of the experiment showed that ETR value increased steadily with a maximum value of 25 and 35 respectively. Measurements of NPQ at the end of the salinity experiments and after dark acclimation period showed similar behaviors for *Porites* under light experiments (Figure 19)



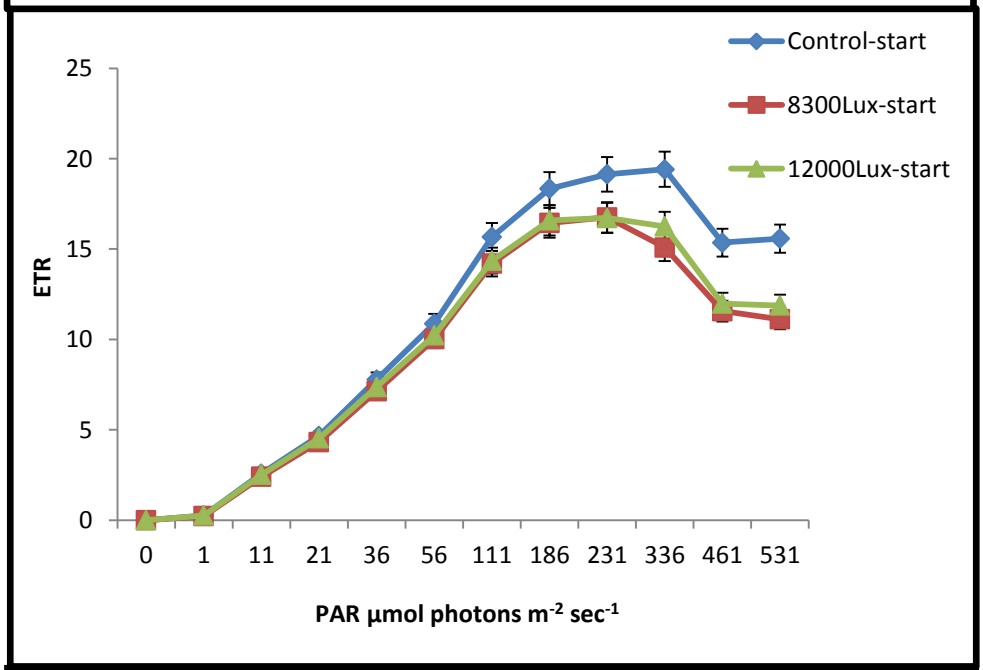


Figure 18. Recorded ETR levels as function of irradiance (PAR) obtained by subjecting *Porites* corals to different light intensity conditions.

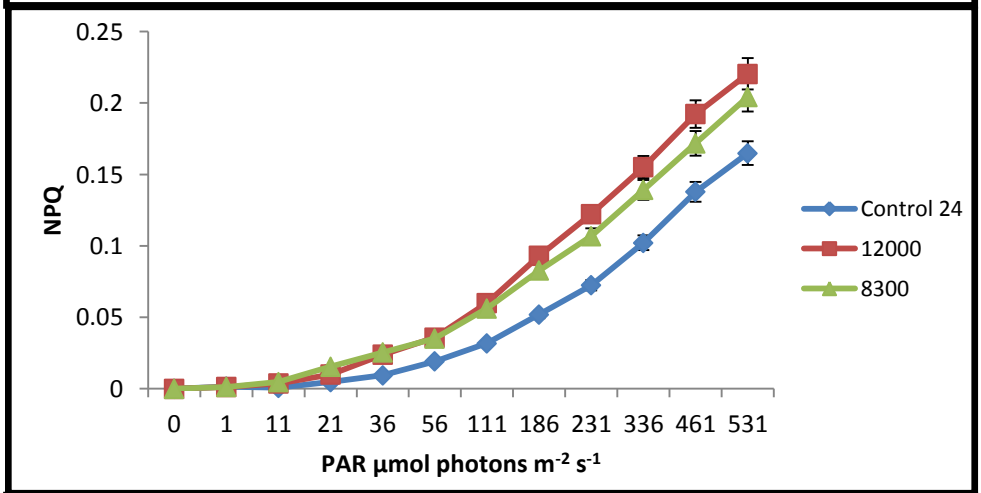


Figure 19. Recorded NPQ levels as function of irradiance (PAR) obtained by subjecting Arabian *Porites* corals to different light intensities conditions.

4.2.3 Images of PAM

PAM images showed no clear differences between 3 coral samples obtained from each tank (Figure 20) All responded similarly to the increased PAR light intensities and maintained photosynthetic efficiency (not saturated) to the end, means able to absorb more light at the end.

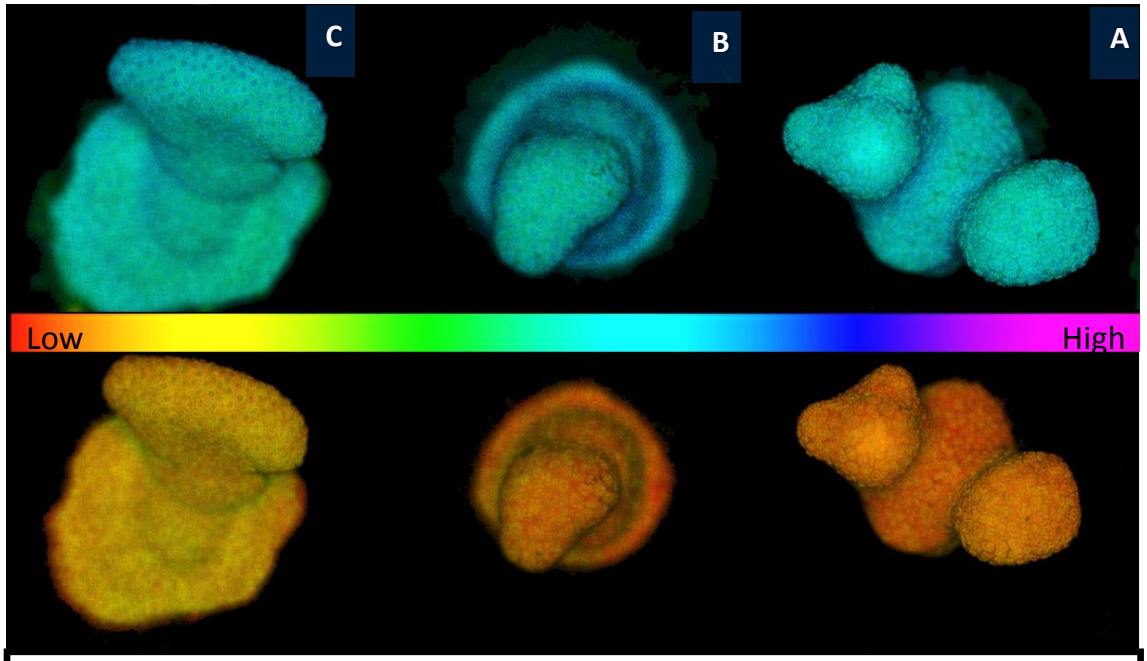


Figure 20. High resolution images of Y(II) at the beginning and at the end of excitation. Control (A), 8300 Lux (B) and 12000 Lux (C)

4.2.4 Changes in Growth Rate

Figure 21 shows close value in all samples, control samples that were maintained at 6800 Lux and treatment samples where light intensity increased to 8000 and 12000 respectively. As shown in Figure 21 control samples the average weight gain was 0.015 gm compared to decrease in average weight in treatment samples that ranged from 0.018 to 0.022 gm.

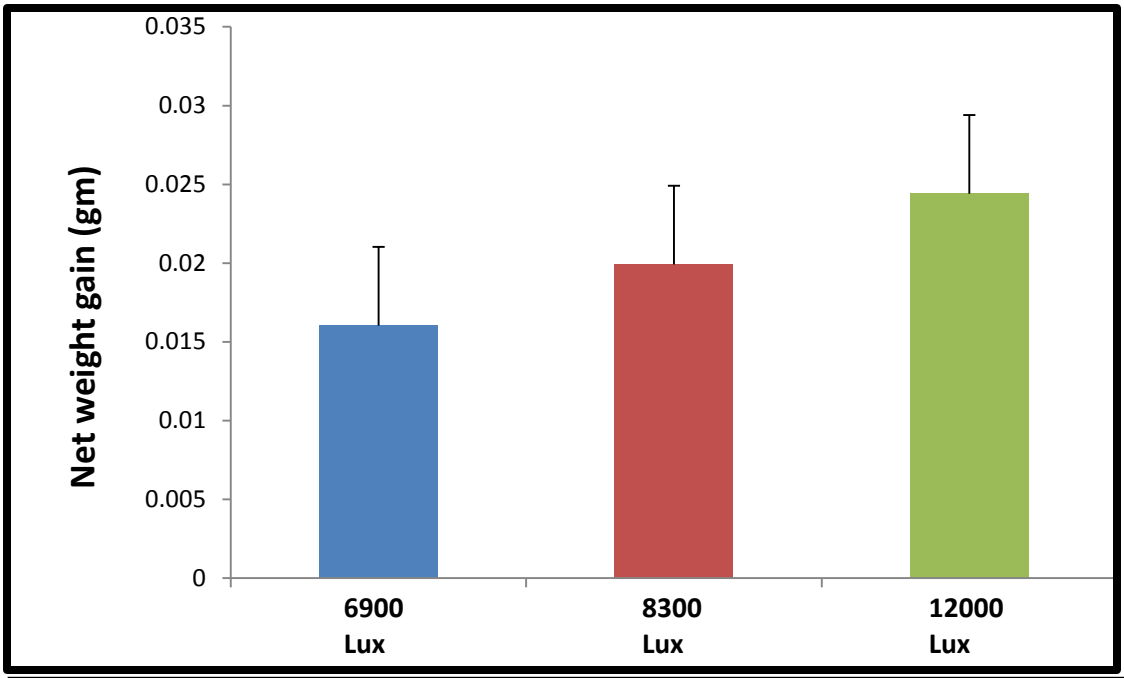


Figure 21. Net weight gain of *Porites* nubbins subjected to different light intensity while keeping all other variables constant

4.2.5 Observation Monitoring

Figure 22 shows the visual assessment of the effect of light intensities on the coral visible features (color, structure, etc.). All corals looked healthy and did not show signs of bleaching.

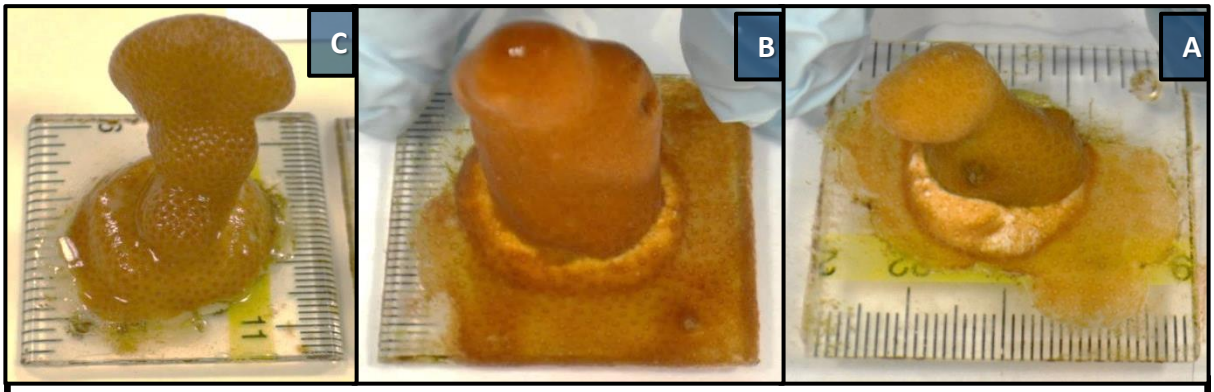


Figure 22. Pictures taken at the end of the experiment of control (A) and experimental units (B-8300 Lux) (C-12000Lux)

4.3 Temperature

The effect of temperature increase on *Porites* nubbins were tested under selected temperatures (control: 24 ±1°C) against two other manipulated temperatures (Treatment 1: 30°C and Treatment 2: 38°C). The photosynthetic activity and growth rate were examined during the experiment.

4.3.1 Maximum Quantum Yield (YII)

After acclimatization the Y (II) values obtained for all nubbins under temperature stress showed same value (0.5 to 0.56) till the third day, after that the Y(II) of the nubbins that was maintained under 38°C started to decline slightly to day six; while other two sets were maintained their yield levels (Figure 23). Following day six, yield of corals under extreme treatment (38°C) started to decline rapidly to reach zero value at day nine. When tested at the end of the experiment; the control nubbins at the (24°C) and treatment-1 (30°C) maintained their normal yield regime the yield was maintained at zero. Nubbins kept at (38°C) showed significant decline in the Y(II) values and reached zero by the 9th day. Samples kept at 24°C and 30°C maintained normal readings and didn't show any differences.

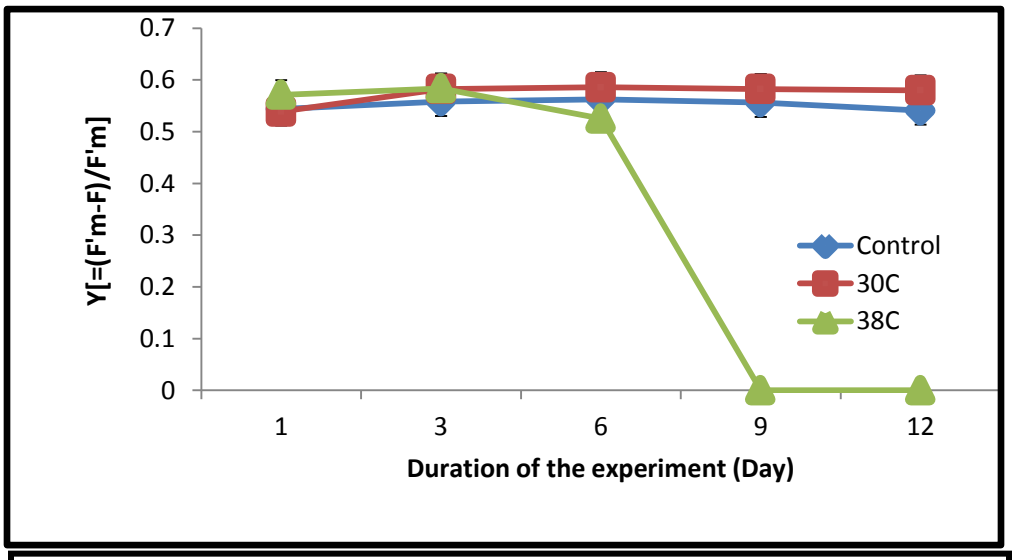
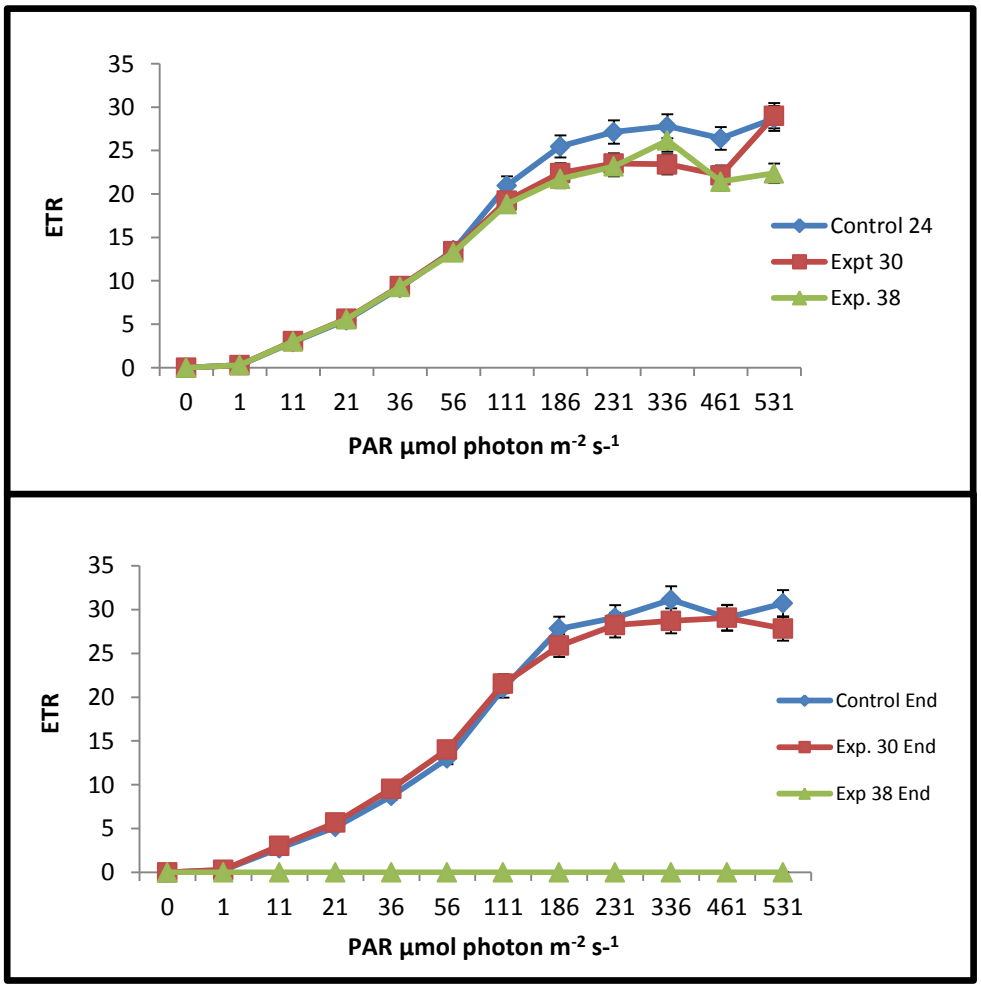


Figure 23. Yield measurements for *Porites Symbiodinium* under three different temperature levels (24, 30 and 38°C)) P <0.05

4.3.2 Rapid Light Curves

The ETR obtained with temperature experiment (Figure 24) showed a steady increase in all samples at the beginning of the experiment reaching a maximum value of 30. However, at the end of the experiment, the control samples and those kept at 30°C reached a maximum of 33 while those kept at 38 did not respond and had values of zero. The NPQ values obtained from Img-PAM represented the response of *Symbiodinium* to elevated and normal temperature levels (24, 30 and 38 °C). The samples that were treated with the highest temperature showed zero NPQ response to all PAR values. A delayed response was detected for the second temperature treatment; where NPQ mechanism was trigged late at 186 µmol photons m^{-2} s^{-1} and only increased to .06; while the control sample exhibited normal onset and elevation of NPQ up to .017



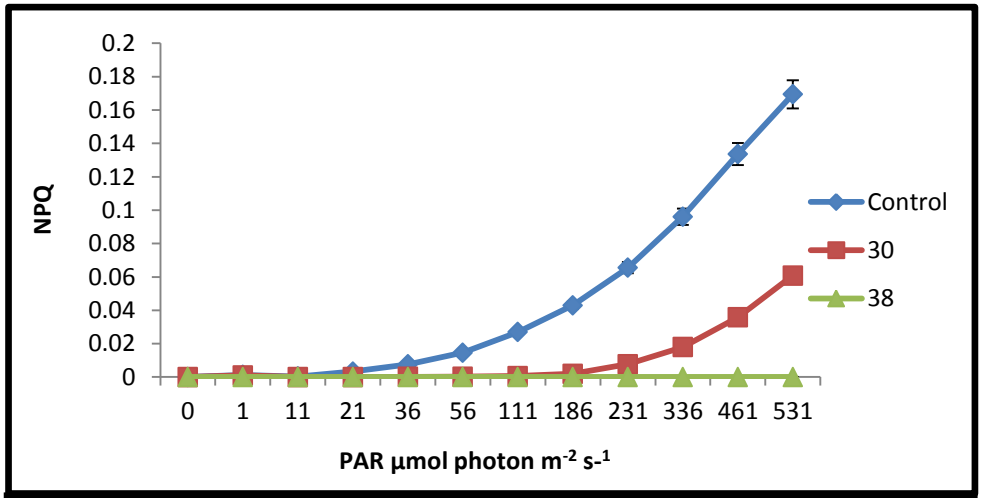


Figure 24. Effect of elevated temperature on ETR and NPQ values of *Porites Symbiodinium*

4.3.3 Images of PAM

Figure 25 indicates the Y (II) at the end of temperature experiment. Photosynthetic efficiency was detected in both control and 30°C, but treatment with 38°C showed zero activity and thus black area.

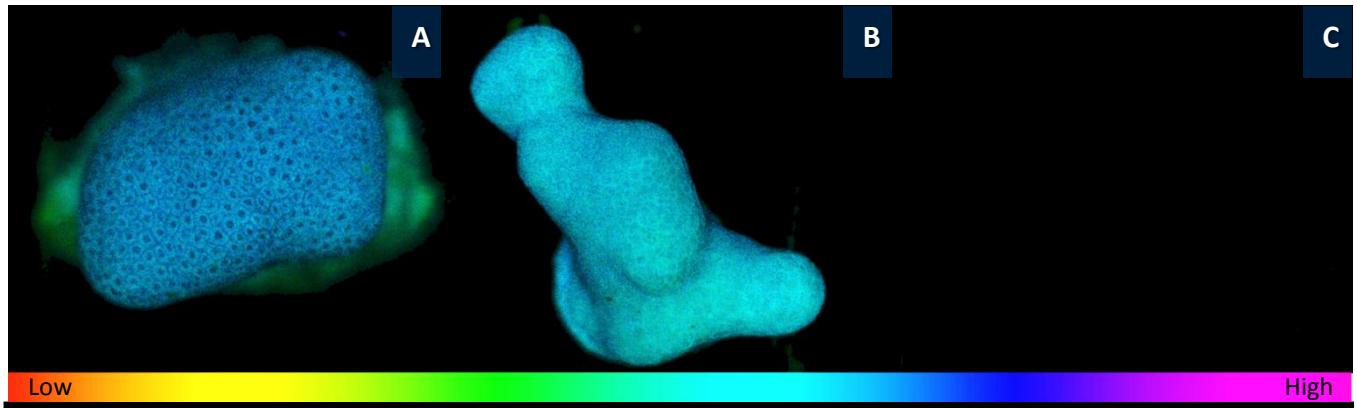


Figure 25. PAM images for temperature experiment- Control (A), 30°C (B), 38°C (C)

4.3.4 Changes in Growth Rate

The gain growth of the nubbins showed significant differences between controls and treatment 3 $p< 0.05$ as shown in Fig. 26. For the control samples, the average weight gain was 0.1 gm

compared to decrease in average weight in treatment 1 and 3 samples by 0.08 and 0.02 gm respectively.

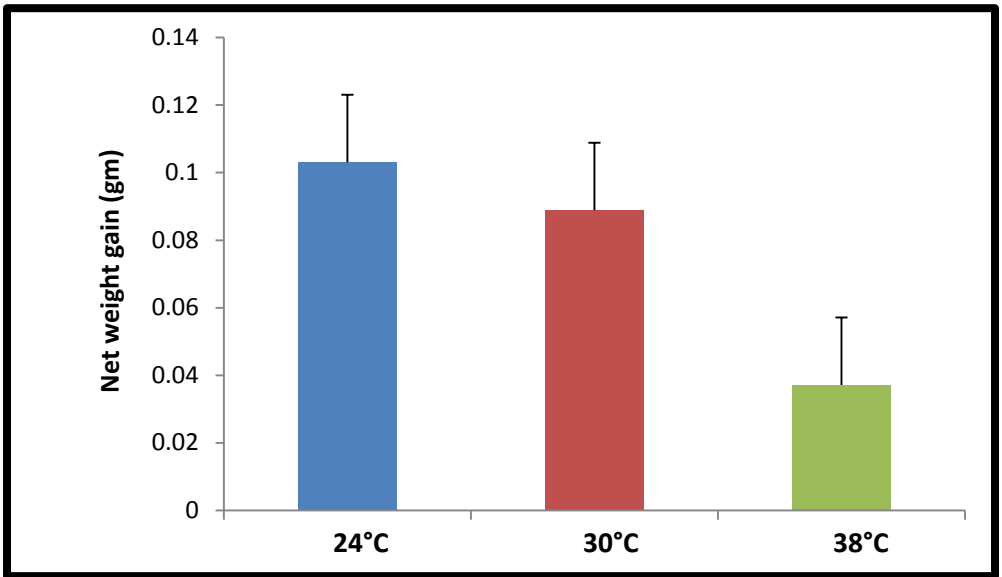


Figure 26. Net weight gain of *Porites* nubbins subjected to different light intensity while keeping all other variables constant

4.3.5 Observation Monitoring

Figure 27 shows the visual assessment of the effect of temperature levels on the coral visible features (color, structure, etc.). Clear bleaching status were observed in the extreme (38°C) temperature treatments.

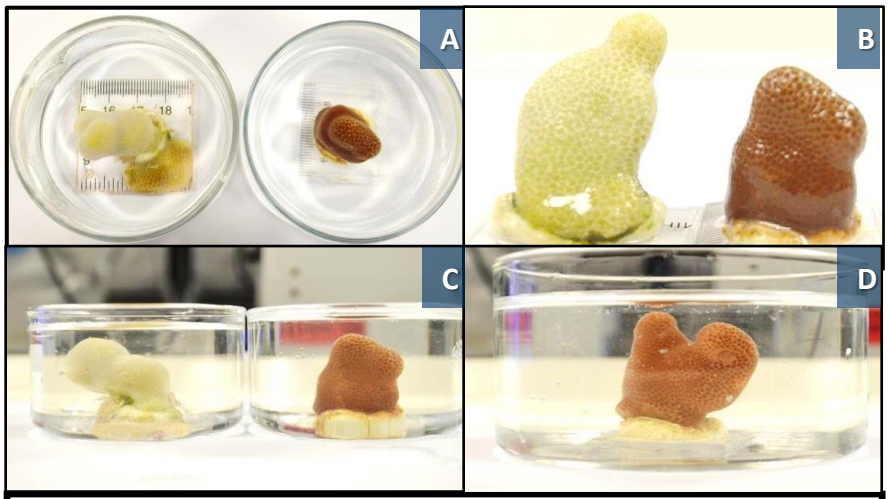


Figure 27. Pictures taken at the end of temperature experiment showing control (D) and experimental units (A, B and C) showing comparison between totally bleached (38°C) and normal appearance

CHAPTER V: DISCUSSION

Understanding the environmental parameters driven impact on coral health under controlled lab system help us to draw a conclusion about corals optimum conditions and tolerance range. My analysis attempted to identify the effect of these stressors on in hospite *Symbidonuim* of *Porites* using Img-PAM and weight gain. Results revealed that elevated levels of temperature and salinity have statistically significant effect on *Symbidonuim* photosynthetic activity; while light intensity did not. High level of salinity (50 psu) affected the corals photosynthetic efficiency by causing a drop to a very low range (0.28) at the end of the experiment thus; affected electron transport rate as well (important in the photosynthetic process). As a result, corals weight dropped down causing partial bleaching which was noticed at day 10 of the experiment with faded spots that covered the corals body and allowing other algae to grow instead. NPQ mechanism was not influenced mainly because it is triggered by high light/ temperature levels.

The decrease in photosynthetic activities of the in hospite *Symbidonuim* under acute salinities and temperature levels were effectively observed through Img-PAM only after the 6th day of exposure. This suggests that in hospite *Symbidonuim* of *Porites* are affected only in relatively durable extreme conditions. A fast and comprehensive recovery of *Porites* health would be therefore more likely to happen for short exposure to extreme temperature or salinities conditions. These findings suggest that *Porites* are relatively resilient to extreme events and appear to have intrinsic features that may help to buffer them against negative effects of the applied extreme conditions (Levas et al., 2013) discussed these possible traits in *Porites* which include: 1- compensating physiological mechanisms, 2- inherent biological traits, and 3- fast recovery rates.

In contrary to salinity, elevated light intensities showed normal regime of efficiency and ETR. Photoadaptive mechanisms of corals allowed them to cope with available light; these mechanisms allow many coral species to maintain metabolic functions over a broad light range (Kleypas, 1999) thus NPQ, as a regulatory mechanism (Lesser, 1997), was activated in response to high light intensity and had higher value (Ryan et al., 2005 and Serodio et al., 2005). The growth rate on the other hand increased with increasing lights.

Finally, *Porites* corals and, in particular, their symbiotic zooxanthellae algae are highly sensitive to increases in temperature above 31°C, where zooxanthellae are ejected and coral bleaching ensues (Brierley and Kingsford 2009). *Symbidonuim* with increased temperature to 38°C exhibited complete death "bleached" at day 6 of the treatment where photosynthetic efficiency (Y), ETR, NPQ dropped to zero value. This decline in photochemical quenching (exposed to bleach) indicates significant loss of functional Photosystem II (PS II) centers under elevated-temperature conditions (Hill et al., 2004). NPQ experienced "chronic photoinhibition" which is irreversible damage to reaction centers of PSII after capacity of protective mechanisms has been exceeded (Lesser 1997). This mechanism was impaired with the extreme temperature levels; where the corals lost this ability to regulate completely. While corals which experienced temperature conditions at 30°C although they show acceptable ranges of Y (II) but they experienced low weight gain and late start in NPQ mechanism which might indicates that chronic photoinhibition process is occurring and the corals are unable to face stress.

CHAPTER VI: CONCLUSION

Salinity, light intensity and temperature are considered the most important ecological factors for corals growth. Using high-resolution fluorocesnse Imaging-PAM combined with its derived detailed image and gain percentage allowed us to identify the stress that is caused by elevated levels of these parameters. High salinities (50 PSU) and temperatures (38°C) affect considerably the photosynthetic activities of the in hospite *Symbidonuim* of *Porites*. Photosynthetic activity is even completely shutoff under 38°C temperatures and an exposure of more than 6 days.

Some corals appeared by physical examination to be normal while they were expressing stress-induced damage that was detected early by PAM before onset of observable indications. For future references, PAM associated to genetic identification tools sound to be very promising to better understand coral mechanism response to different stress stimulis.

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APPENDICES

Salinity Test- Water Quality

Treatment1

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
26.1.14	40	22	8.5	0.025	0.5	0.1	0.02	500
27.1.14	41	23.1	8.5	0.025	0.5	0.05	0.02	600
28.1.14	44	23.6	8.5	0.05	0.5	0.05	0.02	500
29.1.14	42	23.4	8.5	0.05	0.5	0.05	0.02	520
30.1.14	47	23.3	8.3	0.05	0.5	0.05	0.02	740
31.1.14	50	23.2	8.6	0.05	0.5	0.05	0.02	650
1.2.14	50	22.9	8.4	0.05	0.5	0.05	0.02	600
2.2.14	50	22.9	8.3	0.05	0.5	0.05	0.02	550
3.2.14	50	23.1	8.2	0.05	0.5	0.05	0.02	500
4.2.14	50	23.2	8.3	0.05	0.5	0.05	0.02	500
5.2.14	51	23	8.2	0.05	0.5	0.05	0.02	500
6.2.14	52	22.9	8.3	0.05	0.5	0.05	0.02	510
7.2.14	51	23	8.3	0.05	0.5	0.05	0.02	510
8.2.17	50	23	8.3	0.05	0.5	0.05	0.02	500
9.2.14	51	23.3	8.2	0.05	0.5	0.05	0.02	500
10.2.14	51	23.4	8.3	0.05	0.5	0.05	0.02	510
11.2.14	50	23.3	8.3	0.05	0.5	0.05	0.02	500
12.2.14	51	23.4	8.3	0.05	0.5	0.05	0.02	500

Treatment2

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
26.1.14	40	22	8.5	0.025	0.5	0.1	0.02	500
27.1.14	43	23.2	8.4	0.025	0.5	0.05	0.02	600
28.1.14	44	23.7	8.4	0.05	0.5	0.05	0.02	560
29.1.14	45	23.5	8.4	0.025	0.5	0.05	0.02	520
30.1.14	47	23.4	8.2	0.05	0.5	0.05	0.02	720
31.1.14	50	23.4	8.4	0.05	0.5	0.05	0.02	600
1.2.14	50	23	8.3	0.05	0.5	0.05	0.02	600
2.2.14	50	23.1	8.2	0.05	0.5	0.05	0.02	500
3.2.14	50	23.2	8.1	0.05	0.5	0.05	0.02	500
4.2.14	50	23.3	8.3	0.05	0.5	0.05	0.02	500
5.2.14	51	23.1	8.1	0.05	0.5	0.05	0.02	500
6.2.14	51	23.1	8.2	0.05	0.5	0.05	0.02	475
7.2.14	51	23.1	8.3	0.05	0.5	0.05	0.02	500
8.2.17	50	23.2	8.3	0.05	0.5	0.05	0.02	500
9.2.14	51	23.4	8.3	0.05	0.5	0.05	0.02	500
10.2.14	51	23.5	8.3	0.05	0.5	0.05	0.02	525
11.2.14	50	23.4	8.3	0.05	0.5	0.05	0.02	500
12.2.14	51	23.5	8.3	0.05	0.5	0.05	0.02	525

Treatment3

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L	Nitrate mgNO_3/L	Ammonium mgNH_4^+/L	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
26.1.14	40	22	8.5	0.025	0.5	0.1	0.02	500
27.1.14	42	23.2	8.6	0.025	0.5	0.05	0.02	600
28.1.14	44	23.7	8.5	0.05	0.5	0.05	0.02	520
29.1.14	43	23.6	8.5	0.025	0.5	0.05	0.02	580
30.1.14	46	23.5	8.3	0.05	0.5	0.05	0.02	720
31.1.14	49	23.5	8.3	0.05	0.5	0.05	0.02	600
1.2.14	49	23.1	8.3	0.05	0.5	0.05	0.02	650
2.2.14	49	23.1	8.2	0.05	0.5	0.05	0.02	500
3.2.14	50	23.2	8.2	0.05	0.5	0.05	0.02	450
4.2.14	50	23.3	8.3	0.05	0.5	0.05	0.02	500
5.2.14	50	23.1	8.2	0.05	0.5	0.05	0.02	500
6.2.14	50	23	8.2	0.05	0.5	0.05	0.02	450
7.2.14	51	23.2	8.3	0.05	0.5	0.05	0.02	450
8.2.17	50	23.2	8.3	0.05	0.5	0.05	0.02	450
9.2.14	50	23.4	8.3	0.05	0.5	0.05	0.02	520
10.2.14	51	23.4	8.3	0.05	0.5	0.05	0.02	550
11.2.14	50	23.4	8.3	0.05	0.5	0.05	0.02	500
12.2.14	50	23.5	8.3	0.025	0.5	0.05	0.02	500

Control 1

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
26.1.14	40	22	8.5	0.025	0.5	0.1	0.02	500
27.1.14	40	22.8	8.5	0.025	0.5	0.05	0.02	560
28.1.14	40	23.5	8.5	0.05	0.5	0.05	0.02	500
29.1.14	40	23.3	8.4	0.05	0.5	0.05	0.02	580
30.1.14	40	23.2	8.3	0.05	0.5	0.05	0.02	620
31.1.14	40	23.1	8.4	0.05	0.5	0.05	0.02	600
1.2.14	40	22.9	8.4	0.05	0.5	0.05	0.02	600
2.2.14	40	22.9	8.2	0.05	0.5	0.05	0.02	450
3.2.14	40	23	8.2	0.05	0.5	0.05	0.02	400
4.2.14	41	23.1	8.4	0.05	0.5	0.05	0.02	500
5.2.14	41	23	8.3	0.05	0.5	0.05	0.02	450
6.2.14	41	22.8	8.3	0.05	0.5	0.05	0.02	450
7.2.14	41	23	8.4	0.05	0.5	0.05	0.02	450
8.2.17	41	23.1	8.3	0.05	0.5	0.05	0.02	450
9.2.14	41	23.3	8.3	0.05	0.5	0.05	0.02	500
10.2.14	41	23.3	8.3	0.05	0.5	0.05	0.02	450
11.2.14	40	23.3	8.3	0.05	0.5	0.05	0.02	450
12.2.14	41	23.4	8.3	0.05	0.5	0.05	0.02	450

Control 2

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
26.1.14	40	22	8.5	0.025	0.5	0.1	0.02	500
27.1.14	40	23	8.4	0.025	0.5	0.05	0.02	620
28.1.14	40	23.6	8.4	0.05	0.5	0.05	0.02	500
29.1.14	40	23.4	8.3	0.05	0.5	0.05	0.02	580
30.1.14	40	23.4	8.3	0.05	0.5	0.05	0.02	540
31.1.14	40	23.3	8.3	0.05	0.5	0.05	0.02	600
1.2.14	40	23	8.3	0.05	0.5	0.05	0.02	600
2.2.14	40	23	8.2	0.05	0.5	0.05	0.02	450
3.2.14	41	23.2	8.2	0.05	0.5	0.05	0.02	425
4.2.14	40	23.3	8.3	0.05	0.5	0.05	0.02	450
5.2.14	41	23.1	8.2	0.05	0.5	0.05	0.02	450
6.2.14	41	22.9	8.3	0.05	0.5	0.05	0.02	425
7.2.14	41	23.1	8.4	0.05	0.5	0.05	0.02	425
8.2.17	41	23.2	8.3	0.05	0.5	0.05	0.02	425
9.2.14	41	23.4	8.4	0.05	0.5	0.05	0.02	425
10.2.14	41	23.4	8.3	0.05	0.5	0.05	0.02	425
11.2.14	40	23.4	8.3	0.05	0.5	0.05	0.02	425
12.2.14	41	23.5	8.4	0.05	0.5	0.05	0.02	450

Control 3

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
26.1.14	40	22	8.5	0.025	0.5	0.1	0.02	500
27.1.14	40	23.1	8.5	0.025	0.5	0.05	0.02	580
28.1.14	40	23.6	8.4	0.05	0.5	0.05	0.02	580
29.1.14	40	23.6	8.3	0.05	0.5	0.05	0.02	580
30.1.14	40	23.6	8.2	0.05	0.5	0.05	0.02	550
31.1.14	41	23.4	8.2	0.05	0.5	0.05	0.02	600
1.2.14	40	23.2	8.2	0.05	0.5	0.05	0.02	600
2.2.14	41	23.1	8.1	0.05	0.5	0.05	0.02	400
3.2.14	40	23.2	8.1	0.05	0.5	0.05	0.02	450
4.2.14	41	23.4	8.2	0.05	0.5	0.05	0.02	450
5.2.14	42	23.1	8.2	0.05	0.5	0.05	0.02	450
6.2.14	42	22.8	8.1	0.05	0.5	0.05	0.02	500
7.2.14	41	23.2	8.2	0.05	0.5	0.05	0.02	500
8.2.17	41	23.2	8.1	0.05	0.5	0.05	0.02	500
9.2.14	41	23.4	8.2	0.05	0.5	0.05	0.02	500
10.2.14	42	23.4	8.2	0.05	0.5	0.05	0.02	475
11.2.14	40	23.4	8.1	0.05	0.5	0.05	0.02	475
12.2.14	41	23.5	8.2	0.05	0.5	0.05	0.02	450

Light intensity Test- Water Quality

Treatment 1

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
5.3.14	42	23.8	8	0.025	0.5	0.05	0.02	500
9.3.14	40	23.7	8	0.05	0.5	0.05	0.02	510
10.3.14	41	23.7	8	0.05	0.5	0.05	0.02	510
11.3.14	41	24	8	0.05	0.5	0.05	0.02	510
12.3.14	43	24	8	0.05	0.5	0.05	0.02	505
13.3.14	41	24	8	0.05	0.5	0.05	0.02	525
14.3.14	40	24	8	0.05	0.5	0.05	0.02	500
15.3.14	40	24	8	0.05	0.5	0.05	0.02	500
16.3.14	41	24	8	0.05	0.5	0.05	0.02	500
17.3.14	41	24	8	0.05	0.5	0.05	0.02	510
18.3.14	40	24	8	0.05	0.5	0.05	0.02	510
19.3.14	41	24	8	0.05	0.5	0.05	0.02	500
20.3.14	42	24	8	0.05	0.5	0.05	0.02	500
21.3.14	41	24	8	0.05	0.5	0.05	0.02	500
22.314	40	24	8	0.05	0.5	0.05	0.02	500
23.3.14	41	24	8	0.05	0.5	0.05	0.02	500

Treatment 2

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
5.3.14	41	24	8	0.05	0.5	0.05	0.02	500
9.3.14	40	24.1	8	0.05	0.5	0.05	0.02	500
10.3.14	41	24	8	0.05	0.5	0.05	0.02	475
11.3.14	41	24	8	0.05	0.5	0.05	0.02	500
12.3.14	40	24	8	0.05	0.5	0.05	0.02	510
13.3.14	41	23.8	8	0.05	0.5	0.05	0.02	500
14.3.14	40	24	8	0.05	0.5	0.05	0.02	500
15.3.14	40	24	8	0.05	0.5	0.05	0.02	500
16.3.14	41	24	8	0.05	0.5	0.05	0.02	500
17.3.14	40	24	8	0.05	0.5	0.05	0.02	510
18.3.14	41	24	8	0.05	0.5	0.05	0.02	510
19.3.14	40	24	8	0.05	0.5	0.05	0.02	500
20.3.14	40	24	8	0.05	0.5	0.05	0.02	500
21.3.14	40	24	8	0.05	0.5	0.05	0.02	500
22.314	40	24	8	0.05	0.5	0.05	0.02	500

Treatment 3

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
5.3.14	35	24	8.1	0.025	0.5	0.05	0.02	500
9.3.14	40	23.8	8.1	0.05	0.5	0.05	0.02	510
10.3.14	41	23.8	8.1	0.05	0.5	0.05	0.02	515
11.3.14	41	24.5	8.1	0.05	0.5	0.05	0.02	510
12.3.14	42	24.1	8.1	0.05	0.5	0.05	0.02	500
13.3.14	42	23.9	8.1	0.05	0.5	0.05	0.02	510
14.3.14	40	23	8	0.05	0.5	0.05	0.02	500
15.3.14	40	24	8	0.05	0.5	0.05	0.02	500
16.3.14	41	24	8	0.05	0.5	0.05	0.02	510
17.3.14	41	24	8	0.05	0.5	0.05	0.02	515
18.3.14	40	24	8	0.05	0.5	0.05	0.02	500
19.3.14	40	24	8	0.05	0.5	0.05	0.02	515
20.3.14	41	24	8	0.05	0.5	0.05	0.02	500
21.3.14	40	24	8	0.05	0.5	0.05	0.02	510
22.314	40	24	8	0.05	0.5	0.05	0.02	505
23.3.14	40	24	8	0.05	0.5	0.05	0.02	505

Control 1

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
5.3.14	43	24	8	0.025	0.5	0.05	0.02	500
9.3.14	41	23.7	8	0.05	0.5	0.05	0.02	510
10.3.14	42	23.7	8	0.05	0.5	0.05	0.02	510
11.3.14	41	24.5	8	0.05	0.5	0.05	0.02	510
12.3.14	43	24	8	0.05	0.5	0.05	0.02	500
13.3.14	42	24	8	0.05	0.5	0.05	0.02	505
14.3.14	40	24	8	0.05	0.5	0.05	0.02	500
15.3.14	40	24	8	0.05	0.5	0.05	0.02	500
16.3.14	40	24	8	0.1	0.5	0.05	0.02	510
17.3.14	40	24	8	0.05	0.5	0.05	0.02	510
18.3.14	41	24	8	0.05	0.5	0.05	0.02	500
19.3.14	40	24	8	0.05	0.5	0.05	0.02	500
20.3.14	41	24	8	0.05	0.5	0.05	0.02	510
21.3.14	40	24	8	0.05	0.5	0.05	0.02	505
22.314	40	24	8	0.05	0.5	0.05	0.02	510
23.3.14	40	24	8	0.05	0.5	0.05	0.02	500

Control 2

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
5.3.14	37	24	8	0.05	0.5	0.05	0.02	500
9.3.14	40	24	8.1	0.05	0.5	0.05	0.02	510
10.3.14	41	24	8.1	0.05	0.5	0.05	0.02	525
11.3.14	41	24	8	0.05	0.5	0.05	0.02	525
12.3.14	43	24	8	0.05	0.5	0.05	0.02	510
13.3.14	41	24	8	0.05	0.5	0.05	0.02	500
14.3.14	40	24	8	0.05	0.5	0.05	0.02	500
15.3.14	40	24	8	0.05	0.5	0.05	0.02	500
16.3.14	41	24	8	0.05	0.5	0.05	0.02	505
17.3.14	41	24	8	0.1	0.5	0.05	0.02	505
18.3.14	40	24	8	0.05	0.5	0.05	0.02	510
19.3.14	40	24	8.2	0.1	0.5	0.05	0.02	505
20.3.14	40	24	8	0.1	0.5	0.05	0.02	510
21.3.14	40	24	8	0.05	0.5	0.05	0.02	510
22.314	40	24	8	0.05	0.5	0.05	0.02	500
23.3.14	41	24	8	0.05	0.5	0.05	0.02	500

Control 3

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
5.3.14	40	24	8	0.025	0.5	0.05	0.02	500
9.3.14	40	24	8	0.05	0.5	0.05	0.02	510
10.3.14	40	24	8	0.05	0.5	0.05	0.02	520
11.3.14	40	24	8	0.05	0.5	0.05	0.02	510
12.3.14	40	24	8	0.05	0.5	0.05	0.02	510
13.3.14	41	24	8	0.05	0.5	0.05	0.02	510
14.3.14	40	24	8	0.05	0.5	0.05	0.02	510
15.3.14	40	24	8	0.05	0.5	0.05	0.02	510
16.3.14	40	24	8	0.05	0.5	0.05	0.02	510
17.3.14	40	24	8	0.1	0.5	0.05	0.02	510
18.3.14	40	24	8	0.05	0.5	0.05	0.02	510
19.3.14	41	24	8	0.05	0.5	0.05	0.02	510
20.3.14	41	23.9	8	0.05	0.5	0.05	0.02	505
21.3.14	40	23.8	8	0.05	0.5	0.05	0.02	500
22.314	40	23.8	8	0.05	0.5	0.05	0.02	500
23.3.14	41	23.9	8	0.025	0.5	0.05	0.02	500

Temperature Test- Water Quality

Treatment 1

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
24.4.14	41	26.3	8	0.05	0.5	0.05	0.02	400
25.4.14	40	30	8	0.05	0.5	0.05	0.02	400
26.4.14	40	30	8	0.05	0.5	0.05	0.02	400
27.4.14	40	30.9	8	0.05	0.5	0.05	0.02	400
28.4.14	41	30.6	8	0.05	0.5	0.05	0.02	450
29.4.14	40	30.7	8	0.05	0.5	0.05	0.02	420
30.4.14	44	30.7	8	0.05	0.5	0.05	0.02	450
1.5.14	41	30.6	8	0.05	0.5	0.05	0.02	400
2.5.14	40	30.7	8	0.05	0.5	0.05	0.02	400
3.5.14	40	30.7	8	0.05	0.5	0.05	0.02	400
4.5.14	41	30.7	8	0.025	0.5	0.05	0.02	400
5.5.14	41	30.5	8	0.01	0.5	0.05	0.02	400
6.5.14	40	30.5	8	0.01	0.5	0.05	0.02	420
7.5.14	40	30.1	8	0.05	0.5	0.05	0.02	300
8.5.14	40	30.1	8	0.05	0.5	0.05	0.02	400

Treatment 2

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
24.4.14	40	25.7	8	0.05	0.5	0.05	0.02	420
25.4.14	40	29.6	8	0.05	0.5	0.05	0.02	420
26.4.14	40	29.8	8	0.05	0.5	0.05	0.02	420
27.4.14	40	30.2	8	0.05	0.5	0.05	0.02	400
28.4.14	41	29.8	8	0.05	0.5	0.05	0.02	450
29.4.14	41	30	8	0.05	0.5	0.05	0.02	420
30.4.14	44	30.1	8	0.1	0.5	0.05	0.02	400
1.5.14	41	29.8	8	0.05	0.5	0.05	0.02	400
2.5.14	40	30.1	8	0.05	0.5	0.05	0.02	400
3.5.14	40	30.1	8	0.05	0.5	0.05	0.02	420
4.5.14	40	30.4	8	0.025	0.5	0.05	0.02	400
5.5.14	40	29.9	8	0.01	0.5	0.05	0.02	400
6.5.14	41	30	8	0.01	0.5	0.05	0.02	400
7.5.14	40	30.4	8	0.05	0.5	0.05	0.02	300
8.5.14	40	30.2	8	0.05	0.5	0.05	0.02	300

Treatment 3

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
24.4.14	40	26.1	8	0.05	0.5	0.05	0.02	400
25.4.14	40	29.9	8	0.05	0.5	0.05	0.02	420
26.4.14	40	29.6	8	0.05	0.5	0.05	0.02	400
27.4.14	41	31.1	8	0.05	0.5	0.05	0.02	400
28.4.14	42	30.5	8	0.05	0.5	0.05	0.02	450
29.4.14	40	30.4	8	0.05	0.5	0.05	0.02	420
30.4.14	40	30.4	8	0.05	0.5	0.05	0.02	400
1.5.14	41	30.6	8	0.05	0.5	0.05	0.02	400
2.5.14	40	30.4	8	0.05	0.5	0.05	0.02	400
3.5.14	40	30.4	8	0.05	0.5	0.05	0.02	400
4.5.14	41	30.6	8	0.025	0.5	0.05	0.02	425
5.5.14	41	30.5	8	0.01	0.5	0.05	0.02	425
6.5.14	40	30.5	8	0.01	0.5	0.05	0.02	420
7.5.14	41	30.6	8	0.01	0.5	0.05	0.02	450
8.5.14	40	30.6	8	0.05	0.5	0.05	0.02	450

Treatment 4

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
24.4.14	41	38	8	0.05	0.5	0.05	0.02	450
25.4.14	41	37.5	8	0.05	0.5	0.05	0.02	450
26.4.14	40	37.6	8	0.05	0.5	0.05	0.02	450
27.4.14	40	38	8	0.05	0.5	0.05	0.02	450
28.4.14	41	38	8	0.05	0.5	0.05	0.02	400
29.4.14	41	38	8	0.05	0.5	0.05	0.02	400
30.4.14	40	38	8	0.05	0.5	0.05	0.02	450
1.5.14	41	38	8	0.05	0.5	0.05	0.02	400
2.5.14	41	38	8	0.05	0.5	0.05	0.02	400
3.5.14	40	38	8	0.05	0.5	0.05	0.02	400
4.5.14	41	38	8	0.05	0.5	0.05	0.02	400
5.5.14	41	38	8	0.01	0.5	0.05	0.02	400
6.5.14	41	38	8	0.01	0.5	0.05	0.02	400
7.5.14	40	38	8	0.1	0.5	0.1	0.02	300
8.5.14	41	38	8	0.05	0.5	0.6	0.02	300

Treatment 5

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
24.4.14	40	38	8	0.05	0.5	0.05	0.02	400
25.4.14	40	38	8	0.05	0.5	0.05	0.02	400
26.4.14	41	38	8	0.05	0.5	0.05	0.02	400
27.4.14	41	38	8	0.05	0.5	0.05	0.02	400
28.4.14	44	38	8	0.05	0.5	0.05	0.02	400
29.4.14	41	38	8	0.05	0.5	0.05	0.02	400
30.4.14	40	38	8	0.05	0.5	0.05	0.02	450
1.5.14	41	38	8	0.05	0.5	0.05	0.02	400
2.5.14	41	38	8	0.05	0.5	0.05	0.02	400
3.5.14	40	38	8	0.05	0.5	0.05	0.02	400
4.5.14	40	38	8	0.05	0.5	0.05	0.02	400
5.5.14	40	38	8	0.05	0.5	0.4	0.02	400
6.5.14	40	38	8	0.05	0.5	0.5	0.02	400
7.5.14	41	38	8	0.1	0.5	0.6	0.02	300
8.5.14	41	38	8	0.05	0.5	1	0.02	450

Treatment 6

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
24.4.14	41	38	8	0.05	0.5	0.05	0.02	400
25.4.14	40	38	8	0.05	0.5	0.05	0.02	400
26.4.14	40	38	8	0.05	0.5	0.05	0.02	400
27.4.14	41	38	8	0.1	0.5	0.05	0.02	400
28.4.14	41	38	8	0.05	0.5	0.05	0.02	600
29.4.14	40	38	8	0.05	0.5	0.05	0.02	420
30.4.14	40	38	8	0.05	0.5	0.05	0.02	400
1.5.14	40	38	8	0.05	0.5	0.05	0.02	400
2.5.14	41	38	8	0.05	0.5	0.05	0.02	400
3.5.14	41	38	8	0.05	0.5	0.05	0.02	420
4.5.14	40	38	8	0.05	0.5	1	0.02	400
5.5.14	40	38	8	0.05	0.5	1.5	0.1	400
6.5.14	41	38	8	0.05	0.5	1.5	0.1	400
7.5.14	41	38	8	0.05	0.5	1.5	0.4	400
8.5.14	41	38	8	0.05	0.5	1.5	0.4	500

Control 1

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
24.4.14	40	24	8	0.05	0.5	0.05	0.02	420
25.4.14	40	24.4	8	0.05	0.5	0.05	0.02	420
26.4.14	40	24	8	0.05	0.5	0.05	0.02	420
27.4.14	41	24	8	0.05	0.5	0.05	0.02	450
28.4.14	41	24	8	0.05	0.5	0.05	0.02	400
29.4.14	41	24	8	0.05	0.5	0.05	0.02	400
30.4.14	41	24	8	0.05	0.5	0.05	0.02	400
1.5.14	40	24	8	0.05	0.5	0.05	0.02	400
2.5.14	40	24	8	0.05	0.5	0.05	0.02	400
3.5.14	40	24	8	0.05	0.5	0.05	0.02	400
4.5.14	41	24	8	0.025	0.5	0.05	0.02	400
5.5.14	40	24	8	0.01	0.5	0.05	0.02	400
6.5.14	40	24	8	0.01	0.5	0.05	0.02	420
7.5.14	41	24	8	0.01	0.5	0.05	0.02	300
8.5.14	40	24	8	0.025	0.5	0.05	0.02	300

Control 2

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
24.4.14	40	24	8	0.05	0.5	0.05	0.02	420
25.4.14	40	24	8	0.05	0.5	0.05	0.02	400
26.4.14	40	24	8	0.05	0.5	0.05	0.02	450
27.4.14	40	24	8	0.05	0.5	0.05	0.02	500
28.4.14	41	24	8	0.05	0.5	0.05	0.02	400
29.4.14	41	24	8	0.05	0.5	0.05	0.02	400
30.4.14	40	24	8	0.05	0.5	0.05	0.02	400
1.5.14	41	24	8	0.05	0.5	0.05	0.02	400
2.5.14	40	24	8	0.05	0.5	0.05	0.02	400
3.5.14	40	24	8	0.05	0.5	0.05	0.02	400
4.5.14	41	24	8	0.025	0.5	0.05	0.02	400
5.5.14	40	24	8	0.01	0.5	0.05	0.02	400
6.5.14	41	24	8	0.01	0.5	0.05	0.02	400
7.5.14	40	24	8	0.05	0.5	0.05	0.02	400
8.5.14	40	24	8	0.025	0.5	0.05	0.02	300

Control 3

Date	Salinity (‰)	Temp. (° C)	pH	Nitrite (mg NO_2/L)	Nitrate (mgNO_3/L)	Ammonium (mgNH_4^+/L)	Phosphate (mgPO_4/L)	Calcium (mgCa/L)
24.4.14	40	24	8	0.05	0.5	0.05	0.02	400
25.4.14	40	24	8	0.05	0.5	0.05	0.02	400
26.4.14	40	24	8	0.05	0.5	0.05	0.02	400
27.4.14	40	24	8	0.05	0.5	0.05	0.02	400
28.4.14	40	24	8	0.05	0.5	0.05	0.02	400
29.4.14	40	24	8	0.05	0.5	0.05	0.02	400
30.4.14	40	24	8	0.05	0.5	0.05	0.02	450
1.5.14	41	24	8	0.05	0.5	0.05	0.02	420
2.5.14	40	24	8	0.05	0.5	0.05	0.02	400
3.5.14	40	24	8	0.05	0.5	0.05	0.02	400
4.5.14	41	24	8	0.025	0.5	0.05	0.02	400
5.5.14	40	24	8	0.01	0.5	0.05	0.02	450
6.5.14	41	24	8	0.01	0.5	0.05	0.02	420
7.5.14	40	24	8	0.05	0.5	0.05	0.02	300
8.5.14	41	24	8	0.05	0.5	0.05	0.02	300