QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

DEVELOPMENT OF A PROTOCOL FOR CULTURING AND MAINTAINING SEAGRASS, *HALODULE UNINERVIS*, UNDER LABORATORY CONDITIONS AND ITS APPLICATION TO DETERMINE IMPACTS OF LIGHT ON SEAGRASS GROWTH

BY

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ABSTRACT

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Title: Development of a Protocol for Culturing and Maintaining Seagrass, *Halodule uninervis*, under Laboratory Conditions and its Application to Determine Impacts of Light on Seagrass Growth

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Seagrass meadows are an important component of the marine ecosystem as they not only contribute nutrients and organic carbon to the nutrient cycle, but also, provide food, habitat and nursery grounds for a plethora of marine vertebrate and invertebrate species. Several environmental and anthropogenic factors have caused a major decline in their population worldwide. In the Gulf region, the Seagrass is extensively found in the coastal waters and like other marine species, are facing extreme natural stressors, like high temperature and salinity. Additionally, dredging, reclamation, increased eutrophication due to an increase in domestic and industrial discharges as a result of rapid ongoing urban development, such as in Qatar, may impose a threat on the health of the seagrass. These can increase the amount of suspended particles in water and thereby reduce the amount of light reaching the seagrass population. Consequently, their photosynthetic activity can decline and may reach very low levels affecting the rest of the food web that are connected to the seagrass population for either food or habitat. Despite the immense importance of the seagrass neither much is known about its ecology nor about its association and dependence on the abiotic factors. This study was designed to investigate the possibility of maintaining indigenous seagrass species under laboratory conditions so as to be able to understand its ecology and requirements for providing a sustainable population. Furthermore, this study investigated the impact of three light intensities on the health of seagrass held at a constant temperature of 22^{o}C for about two months. Pulse Amplitude Modulated (PAM) fluorometry was used to assess the maximal quantum yield (YII) which is the photosynthetic response of seagrass to various light intensities. The study demonstrated that it is possible to maintain and achieve growth in seagrass population under controlled laboratory conditions. The PAM measurements showed that the growth of seagrass is dependent on the amount of light received. A total lack of light led to a 66% decline in YII while a light at 227 PAR (µmolm^{-2}s^{-1}) enabled the seagrass to maintain its photosynthetic ability as seen in the field. A further increase in light (452 PAR) increased the photosynthetic function only slightly.

DEDICATION

I would like to dedicate my work to my mother for without her constant support, motivation, inspiration and enthusiasm none of this would have been possible.

ACKNOWLEDGMENTS

At the outset, I would like to thank God who gave me the knowledge, fortitude and perseverance, and carried me through my studies. I would like to take this opportunity to thank everyone who helped and supported me. I am deeply grateful for my family, especially my mother, for her unconditional support and patience for me to complete my work. I would like to thank my supervisor and committee members, Dr. Jassim A Al-Khayat, Dr. Fahad Al-Jamali, Dr. Yoursia Soliman, and Dr. Suhur Saeed for their unlimited patience and knowledge in guiding me through this journey in the magnificent world of science and in the continuation of my education. I wish to give special thanks and appreciation to Dr. Nandita Deb, Mr. Ismail Al-Sheikh and Mrs. Ruqaiya Yousif for their support in the field, in the laboratory as well as in mentoring me in this process. Without their support this research work could not have been completed successfully. I would also like to give thanks to Dr. Fahad Al-Jamali and his team for their invaluable help with the collection of the seagrass. I appreciate their help and hospitality in accommodating my specific needs for this project. I greatly appreciate the opportunity, and facilities provided by ExxonMobil Research Qatar for conducting this research work and I would like to thank them for taking interest in my work and extending their constant support to complete this project.

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CHAPTER 1: INTRODUCTION

1.1 Seagrass meadows and their importance

Seagrass meadows are considered as highly important marine ecosystem engineers. Despite their ecological significance in the aquatic ecosystem, their populations are declining globally (Ahmad-Kamil *et al.*, 2013; Waycott *et al.*, 2009). There is a lack in research of these highly important ecosystems worldwide, more so, in the Gulf region. In the Arabian Gulf, some of the most extensive seagrass beds occur around the Qatari peninsula (Vaughan *et al.*, 2019), and are extremely important to the local marine environment. Thus, it is imperative to understand the ecological role played by seagrass and the factors that could limit its growth and distribution in the marine ecosystem. To that goal, this study is an important contribution in understanding the ecological need of the indigenous seagrass population, in particular, the role of light in the sustenance of seagrass.

Seagrass beds play a vital role due to the vast services they provide to the marine ecosystem. They have large productivity levels, stabilize the sea bottom, and provide food, habitat and nursery grounds for numerous vertebrate and invertebrate species. According to Short *et al.* (2011) seagrass provide nutrients (P and N) and organic carbon to many parts of the ocean including the deep sea, and they contribute significantly to carbon sequestration. Furthermore, there is a vast range of biodiversity which depends on seagrass meadows to meet specific dimensions of their niche. These seagrass beds are of crucial importance to support the second largest population of Dugongs in the world (Preen, 2004). The sensitivity of seagrass to changes in water quality plays an important role as their health can be an indicator of the overall health and functioning of coastal ecosystems (Larkum *et al.*, 2006). They also provide support to the local economies by means of ecotourism and commercial and recreational fishing activities.

1.2 Threats faced by seagrass meadows

The huge biological, economical and attractive values which seagrass has does not mean the threats faced by them are any less. It was estimated that in 2010 the value of ecosystem service by seagrass was $34,000 US per hectare per year (Short *et al.*, 2011). It is estimated that globally 15% of seagrass species are threatened (Short *et al.*, 2011) and seagrass meadows have declined worldwide at a rate of $110 km^2 \, yr^{-1}$ between 1980 and 2006 (Waycott *et al.*, 2009). The main cause for threat is the anthropogenic activities. Globally seagrass meadows are located in shallow inshore waters up to a maximum depth of approximately 70m (Grech *et al.*, 2012). In the Arabian Gulf however, most meadows are located in less than 10 m deep water, therefore their health and survival can be strongly influenced by complex natural and human activities in and around the coastal areas. The greatest threat faced is that of urban/industrial runoff, urban/ port infrastructure development, agricultural runoff, brine water discharge from desalination plants, and dredging activities taking place along the coast (Al-Wedaei *et al*., 2011).

Primarily seagrass degradation is due to a reduction in water clarity caused by increased turbidity and nutrient loading (Erftemeijer *et al.*, 2006). In many cases there is directly or indirectly a loss of seagrass vegetation due to dredging. As mentioned by Erftemeijer *et al.*, (2006) in Tampa Bay, Florida, a loss of approximately 81% of seagrass was due to a combination of increased eutrophication from nutrients in domestic and industrial discharge and increased turbidity and removal or burial during dredging. The impact these activities have on seagrass is a reduction of light. Since light plays an important role in primary production, any alteration in the light intensity reaching the seagrass could drastically disturb the carbon-budget affecting the carbon available and consequently reduce photosynthetic carbon-fixation. This can have a major effect on seagrass health and biomass production (Collier *et al.,* 2011; McMohan *et al.*, 2013).

1.3 Seagrass anatomy and physiology

Seagrasses are a paraphyletic group of marine hydrophilus angiosperms, which has evolved three to four times from land plants back to the sea (Papenbrock, 2012). They are abundant in estuaries and marine environment. They do not grow in salinities less than 18 psu or fresh water. They have specialized pollen to propagate underwater; they have seeds that are dispersed through both abiotic (e.g. water current) or biotic (e.g. carried by marine mammals) factors in the sea. The specialized leaves of seagrass have reduced cuticles and lacks stomata. The epidermis is the main site for photosynthetic activity. The seagrass have extensive roots that helps it to anchor into the seabed. They have horizontal rhizomes/ stems for mechanical support so as to not get washed away with strong currents.

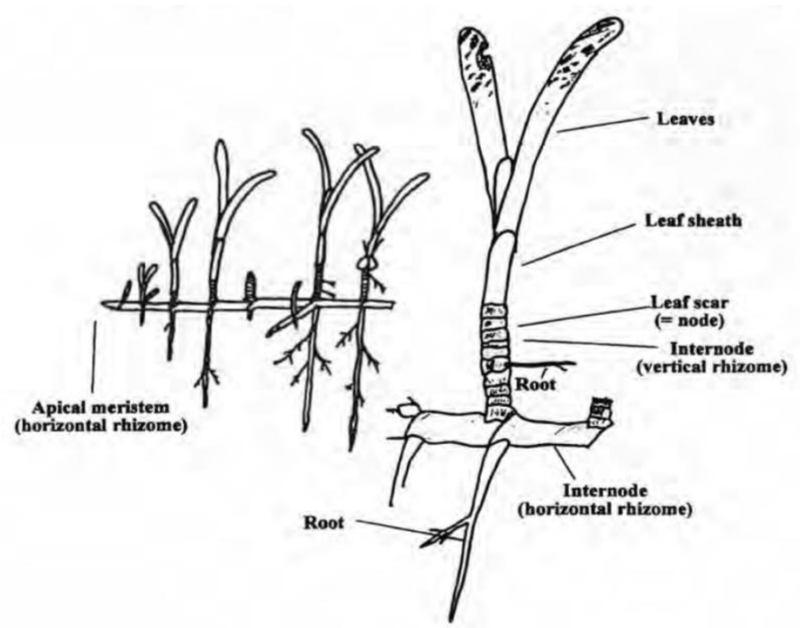


Figure 1: Diagram of the basic components of seagrass architecture. (Hemminga & Duarte, 2000).

1.4 Method for analyzing the seagrass response to light stress

1.4.1 Pulse-Amplitude-Modulation (PAM) fluorometer

In order to determine the health of seagrass it is important to get an understanding of its photosynthetic activity by measuring the chlorophyll content in its leaves. Seagrass being a flowering plant requires to photosynthesize in order to survive. Through many different research studies in this field an instrument called a Pulse Amplitude Modulated (PAM) fluorometer (Figure 2) was developed to measure the chlorophyll fluorescence of photosystem II.

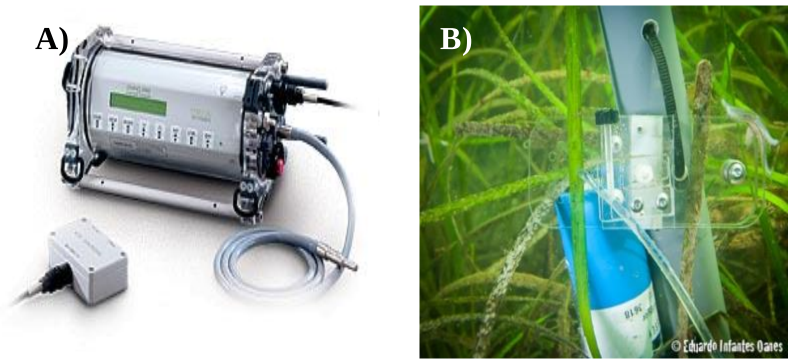


Figure 2: A) Diving PAM, B) A close up of the measuring gun on the leaf

This instrument uses a rapid in situ technique to measure the maximal optimal quantum yield (YII) of photosystem II of the seagrass. According to Silva *et al.* (2009), PAM fluorometer emits continuous short measuring-light pulses of red or blue light. As the fluorescence signal caused by this measuring light is captured during the very short pulse periods, external disturbances, background signals and transient artefacts are to be eliminated to avoid masking the fluorescence signals. In PAM fluorometers, the short pulses of measuring light induce the emission of a fluorescence signal. When a saturating light pulse of about 0.8s duration is applied to the plant sample, all reaction centers become reduced (or 'closed') and the fluorescence emission becomes maximal. PAM fluorometers, the more recent type, emit continuous short measuring-light pulses of red or blue light. This instrument allows measurements to be conducted in full sunlight, due to a special emitter-detector unit that separates the fluorescence signal from ambient light (Silva *et al,* 2009). Most PAM fluorometers are portable and one model, the Diving-PAM (Figure 2), which was used in the present study, is adapted for underwater operation. The mid-portion of each leaf (3cm from meristem) should be held in a leaf clip (Walz, DIVING LC) and fluorescence measurements are to be made underwater with the light probe joined to the leaf clip. A weak pulsed red light (< 1 μmol quanta m^{-2} s^{-1}) would be applied to determine the fluorescence yield in an illuminated state. A saturating pulse (800ms of 8000μmol quanta m^{-2} s^{-1} PAR) would then be applied. The change in fluorescence caused by the saturating pulse in relation to the maximal fluorescence is a measure of quantum yield (Campbell *et al.,* 2006). Along with the maximum quantum yield, the diving PAM instrument can be used to process many other analysis of photosynthesis.

1.5 Status of the seagrass in the Arabian Gulf

The Arabian Gulf is a semi-enclosed sea that is very shallow with an average depth of 35m. There is very limited water exchange with adjacent basins and it has high evaporation rate that results in high sea temperature ranging from 21 to 34°C and high salinity from 37 to 44 psu (Taher *et al.,* 2012). A combination of these elements result in very harsh conditions for this ecosystem. According to Siebold (1973) due to the high turbidity in the Arabian Gulf, the photic zone only extends up to 6 -15 meters. There are only four species of seagrass found in the Arabian Gulf; namely, *Halodule uninervis* (forms more than 90 percent of the sea grass population), *Halophila stipulacea*, *Halophila ovalis and Syringodium isoetifolium* (Phillips, 2003). These species are able to tolerate extreme temperatures and salinity enabling them to survive in the Arabian Gulf. Seagrass beds are distributed along most of the shores of the Gulf. According to Erftemeijer & Shuail (2012), there is around 7000 km^2 of seagrass that have been mapped up until 2012, of which the largest seagrass beds occur off the coast of United Arab Emirates and between Qatar and Bahrain. Seagrass beds have a heterogeneous distribution and are often interspersed with macroalgal beds and sandy sections (Basson *et al*., 1977; Sheppard *et al*., 1992).

CHAPTER 2: LITERATURE REVIEW

Seagrass plays a fundamental role in the ecosystem, therefore understanding their ecology in terms of the abiotic factors, such as light, required for their optimum growth and survival are vital. Seagrasses are submerged vascular plants that grow in shallow marine and estuarine environments. Due to their large mass of belowground roots and rhizomes, they need the highest amount of light among all plants, requiring up to 30% of full surface-incident sunlight (Fourqurean *et al*., 2003). Knowledge of the relationship between photosynthetic ability and water quality parameters, like chlorophyll concentration and turbidity, has been used all over the world to predict light penetration in the water column and to define the water quality limits for optimal survival of submerged aquatic vegetation.

It is vital to understand such parameters and the role they play in this environment as seagrasses have not been studied in depth in the Qatari marine ecosystem. The various roles of seagrass as a highly productive ecosystem, for sustaining aquatic communities, for influencing fish production, for providing habitat for fish, shellfish and providing appropriate grounds for fish hatchling development in the harsh conditions found in the marine environment in the Arabian Gulf has been well documented (Erftemeijer and Shuail, 2012). Moreover, they perform important physical functions such as filtering coastal waters, dissipating wave energy, anchoring sediments and has a crucial role in the nutrient cycles as shown below in Figure 3 (Mazarrasa *et al.*, 2018). Although they are highly valuable ecologically and economically, many seagrass habitats around the world have been completely destroyed or are now in rapid decline, primarily due to eutrophication or turbidity from industry, dredging or catchment run-off, as well as due to natural disturbances. Terrestrial input and dredging, which re-suspends fine sediments, are two major processes that declines light in the benthic zone.

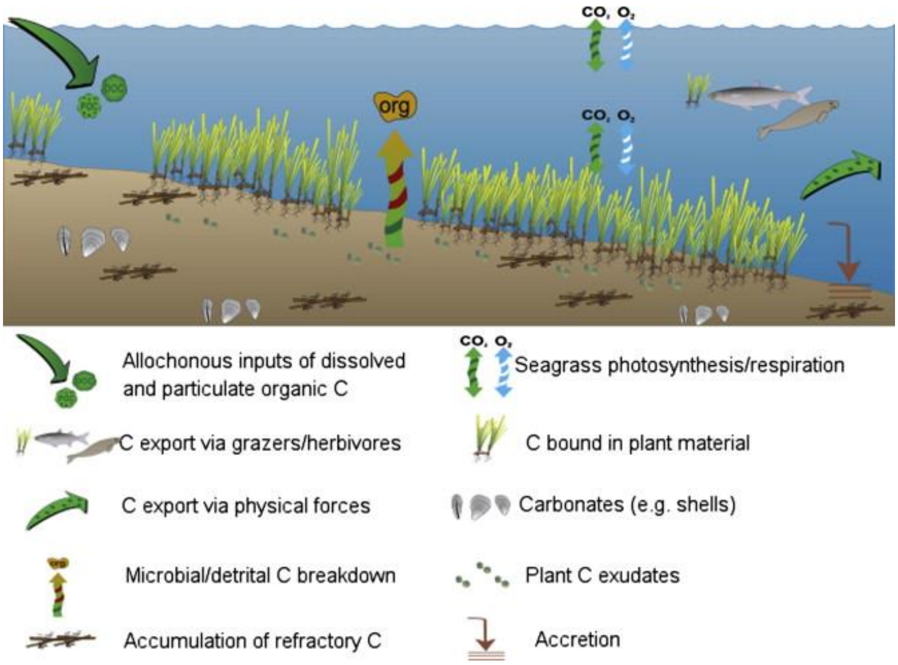


Figure 3: Demonstration of carbon cycling and the importance seagrass plays in the cycle (Mazarrasa *et al.*, 2018)

Seagrasses can acclimate to changing light levels but under extreme reductions in light availability, photosynthetic carbon fixation is directly and drastically reduced (Collier *et al.*, 2016). Reductions in light can be a limiting factor which de-stabilizes seagrass carbon budgets (Collier *et al*., 2011) and limits the amount of carbon needed for appropriate growth and biomass production. Due to the ecological importance of seagrass as food, habitat and carbon sequestration (Collier *et al.,* 2016), any alteration in growth and biomass of seagrass induced by light limitation are a major concern in risk assessment and environmental management. It is essential to understand the light requirement for optimum seagrass survival to prevent any seagrass decline in ecosystems where they form an important link in the food web, such as in Qatar.

Some studies have demonstrated how light effects the growth of seagrass in marine environments around the world. Some of those results are shown below and can be used as a reference to compare to the local environment in Qatar. A recent study, conducted in the Great Barrier Reef area, provides the threshold for morphological responses to light reduction for four tropical seagrass species (Collier *et al*., 2016, Figure 4). The authors studied the morphological response (shoot density and growth) of four Indo-West Pacific seagrass species (*Cymodocea serrulata*, *Halophila ovalis*, *Halodule uninervis* and *Zostera muelleri*) to six daily light levels ranging from 0 to 23 mol m^{-2} d^{-1} (0–70% surface irradiance) in cool (~23 °C) and warm temperatures (~28 °C) over 14 weeks. The response was higher at 28 °C than at 23 °C and was more pronounced for *Z. muelleri* and *H. ovalis* than for *C. serrulata* and *H. uninervis*, for both the time taken for low light treatment to take effect and the predicted time to shoot loss (e.g. 17–143 days at 0 mol m^{-2} d^{-1}). Potential light thresholds that maintained 50% and 80% of seagrass shoot density fell within the ranges 1.1–5.7 mol m^{-2} d^{-1} and 3.8–10.4 mol m^{-2} d^{-1}, respectively, depending on temperature and species (Collier *et al*., 2016).

Number of weeks until the effect of light treatment on shoot density and growth rate was significant (one-way Anova, $p<0.05$) for cool and warm temperatures

Species	Weeks until shoot density significantly affected by light treatment		Weeks until growth rate significantly affected by light treatment	
	Cool	Warm	Cool	Warm
C. serrulota	>14[a]	4-7	4-7	1-4
H. uninervis	10-14	4-7	1-4	<1
Z. muelleri	0-4	0-4	4-7	<1
H. ovalis	4-7	1-4	1-4	1-4

[a] Shoot density of C. serrulota in cool temperatures was not significantly affected by light treatment over the duration of the experiment (14 weeks)

Figure 4: Table showing data from experiments done by Collier *et al*., (2016)

This table by Collier *et al*. (2016) shows the length of time it took seagrass of different species to show a decline in growth under different light intensities. The figure below shows their findings for *Halodule uninervis,* the species chosen for the present study.

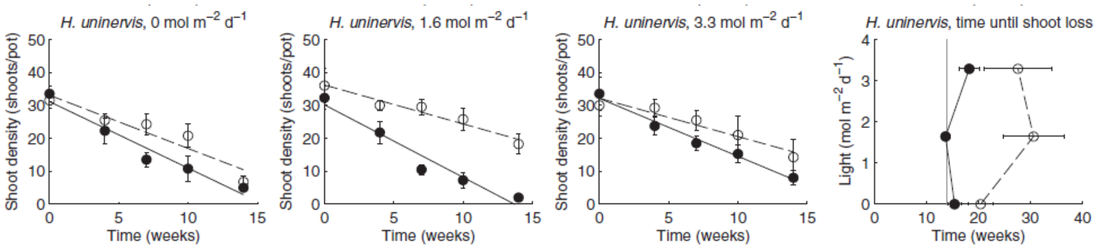


Figure 5: Shoot loss trajectories for *H. uninervis* at light conditions of 0-3.3 molm^{-2}d^{-1} and fitted linear functions in cool (dashed lines, open circle) and warm (solid lines, closed circles) temperatures, error bars indicate ±SE (Collier *et al*., 2016)

These results clearly show the effect of light on seagrass. Therefore, they can be a good source of reference for comparing to results that would be generated in this study on seagrass, *Halodule uninervis,* from the Qatari environment.

According to Short *et al.*, (2001) there are different light requirements for different species of seagrass and therefore the seagrass distribution is dependent on the availability of light intensity needed for that specific species to survive and grow. Some species of seagrass are intolerant to high light intensities and show photoinhibition in shallow intertidal zones (Short *et al.*, 2001). These species perform best in deeper areas of the sea. In contrast, some grow best in high light intensities.

A study conducted in 2007 in Australia (Bité *et al.*, 2007) while examining the Chlorophyll fluorescence measures of seagrasses *Halophila ovalis* and *Zostera* *capricorni* revealed differences in their response to experimental shading. The study used Pulse Amplitude Modulated (PAM) fluorometry to record changes in the photosynthetic activity of seagrass along natural gradients in light. They found that in both species tested, as shading increased, the photosynthetic variables significantly ($P < 0.05$) decreased by up to 40% for maximum electron transport rates (ETR_{max}) and 70% for saturating irradiances (E_k). The photosynthetic efficiencies (α) and effective quantum yields ($\Delta F/Fm'$) increased significantly ($P < 0.05$), in both species, for 90% shaded plants compared with 0% shaded plants. These responses to changes in light propose that photosynthetic variables can be used to rapidly assess the status of seagrasses when subjected to either sudden or prolonged duration of reduced light (Bité *et al.*, 2007).

The primary limiting factor in seagrass growth is often light availability. Light availability is also coupled with water turbidity. An increase in water turbidity have been noted over the years in the Qatari waters which could be related to an increase in anthropogenic activity in the region, particularly, around the coast. With the rapid expansion, there is an increase in coastal construction and dredging activities. These activities may have the potential to have some impact on the environment and in particular on the seagrass meadows which sustain all forms of life including that of the endangered Dugong and Green turtle. In order to protect these areas, it is vital to understand seagrass ecology and essential requirements needed to flourish.

2.1 Research Objectives:

The specific objectives of the present study are:

- To establish a protocol for culturing and maintaining seagrass under controlled laboratory conditions.
- To evaluate effects of varying light intensities on seagrass growth.

CHAPTER 3: METHODS

3.1 Research Strategy

Only one species of seagrass, *Halodule uninervis,* was chosen for this study. This species is the most abundant type found in Qatari waters. It was exposed to three different light intensities in the laboratory set up. It was maintained in glass tanks, under controlled conditions, to evaluate its physiological responses, more specifically photosynthetic responses, to reduction in light intensity. The expected response to persistent reduced light intensity were the reduction in the photosynthetic activity of the seagrass, leading to a reduction in growth and ultimately leading to their death.

The primary aim was to establish a protocol for culturing and maintaining seagrass under controlled laboratory conditions. The second aim was to investigate the effects of varying light intensities on seagrass growth. To achieve these objectives, light stress study was designed and carried out over a period of 56 days after allowing a two-week period of acclimatization at equal light intensity for all tanks.

Three sets of light intensity ranges were chosen for this experiment: low light (no light set up; 0 PAR ($\mu molm^{-2}s^{-1}$)), medium light (two lights set up; 227 PAR) and high light (three lights set up; 452 PAR), with two replicates for each treatment. The water quality and chemistry were monitored and maintained on a weekly basis and kept as constant as possible throughout the duration of the experiment. For the assessment of the photosynthetic activity of the seagrass the diving-PAM (Pulse Amplitude Modulation) was used.

3.2 Seagrass Sampling and Transportation

Seagrass samples were collected off the coast of Al Aaliya Island, Qatar (coordinates in decimal degree 25.3867100 N, 51.5906710 E; Figure 6). The seagrass collection was performed by expert divers specialized in the collection and translocation of seagrass. The seagrass species, *Halodule uninervis,* were selected due to the fact that it is the most abundant species in the Qatari marine ecosystem, in particular, and in the Gulf water, in general. The area where the seagrass were collected was chosen due to the dense seagrass meadows found in that area as indicated with the red circle in Figure 6.

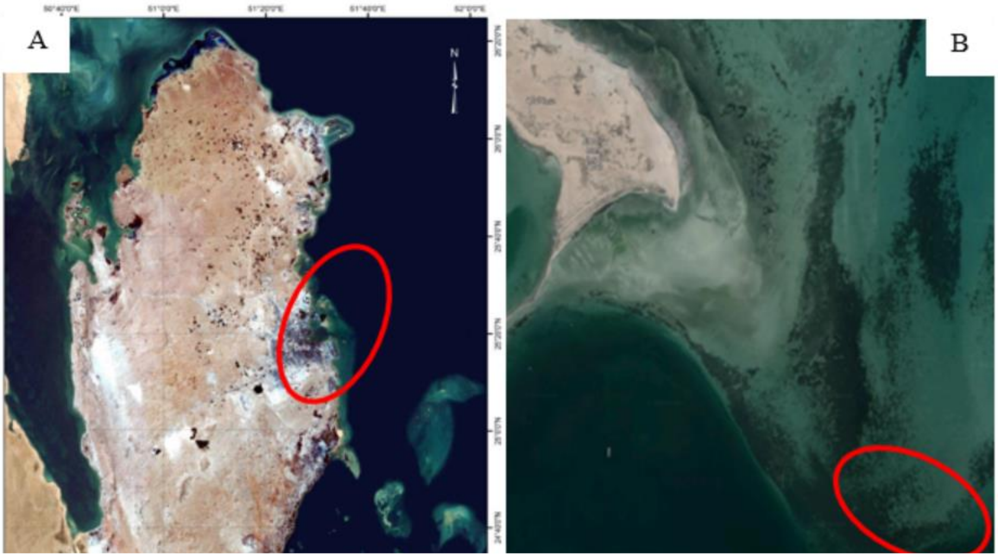


Figure 6: A) Map of Qatar B) Map showing seagrass distribution South East of Al Aaliya Island. Image by Esri GeoEye 2017

The seagrass were collected using several 30 X 30 cm scoop, specially designed by Dr. Fahad Al-Jamali and team, for seagrass collection and translocation.

Once collected, the seagrass were placed in a wide container, partially submerged in sea water from the sampling site and transported to the laboratory at ExxonMobil Research Center (EMRQ) in Qatar where the experiments were to be conducted. Figure 7 shows the transportation containers with the sampling scoops filled with seagrass and seawater. The seagrass were transported in hard, open top, plastic containers partially filled with seawater (Figure 7) from the collection site and delivered to the laboratory within ~2h of sampling.

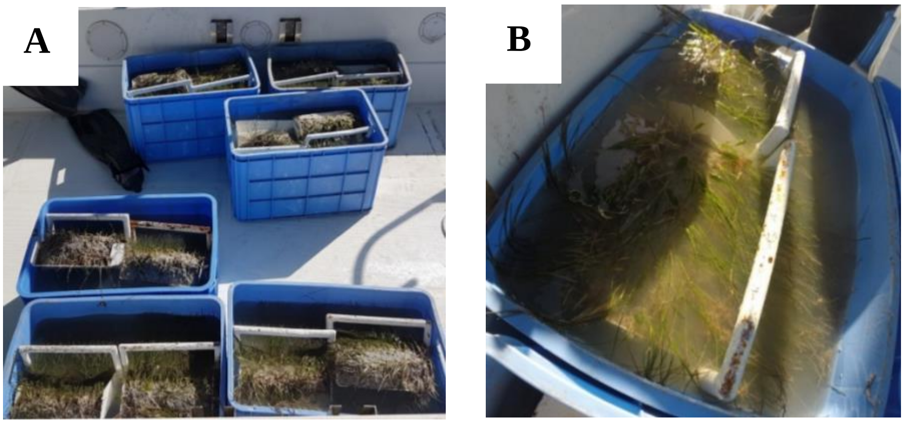


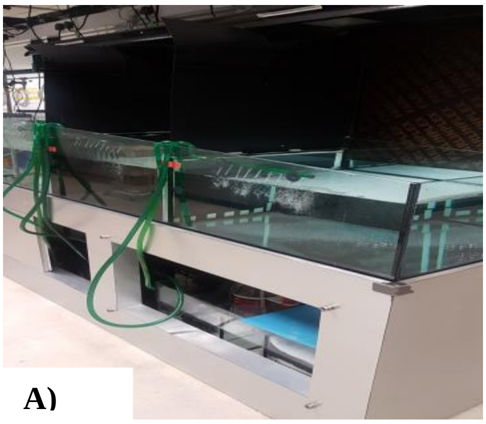
Figure 7: A) the open containers with the scoops filled with seagrass B) a close up image of the collected seagrass ready for transportation.

3.3 Seagrass Acclimatization

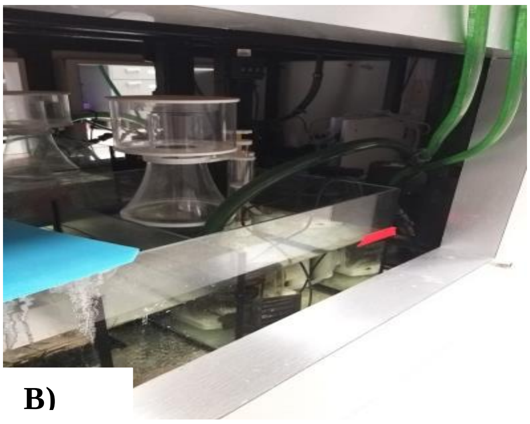
The seagrass quadrants were distributed among 6 laboratory glass tanks, four quadrants per tank. One scoop at a time was gently lowered inside the tank filled with artificial sea water at 40 psu. The scoop was gently pulled out leaving behind the seagrass embedded in the sand quadrant at the bottom of the glass tank. This process was done very slowly so as to minimize any disturbance and release of suspended particles from the sand bed. This avoided any undesirable increase in turbidity and settling of suspended particles on the leaf blades. Likewise, this process was repeated for six tanks, with four scoops per tank. The initial seawater parameters were measured and were maintained at 40 psu and water temperature 22 °C throughout the experimental duration in order to reproduce as much as possible the original conditions at the sampling site. The seagrass embedded in the sand substrate were allowed to sit in the tank for 24 hours, undisturbed. After this, all organisms, such as crabs, shrimps, sea stars etc. were hand-picked and removed from the tanks. Each tank, for two weeks, was provided with 124 PAR light intensity through one light source at 12h L: 12h D photoperiod during the acclimation period.

3.4 Physico-chemical set up and the maintenance of tanks

All tanks were filled with artificial seawater prepared by mixing commercially available aquarium sea salt and demineralized water before collecting seagrass. Each tank was 60 X 68 X 39 cm (WxLxH) and each was filled with 160 L of artificial seawater. Water salinity and chemistry were maintained and monitored to mimic natural seawater of the collection site. Water chemistry parameters (nitrate, nitrite, ammonium, phosphate, TOC) were measured once a week using a JBL© water test kit, Germany and SKALAR TOC/TN analyzer, Switzerland, respectively. pH was measured using a HACH pH meter. Salinity was measured using a handheld refractometer and maintained at 40 psu. Temperature was measured using a thermometer which was placed in each tank. Figure 9 shows the set-up of the tanks. As is seen in the figure, the tanks were connected to a biological filtration unit (placed at the bottom) to maintain water quality. Three tanks were connected to one single filtration unit, by water cascading and recirculating through the three tanks.



A)



B)

Figure 8: A) the set-up of glass tank with black dividers for light B) set-up of the main bio-filtration unit.

Each tank had additional two External Canister filtration units (EHEIM 350, Germany) with their outlets placed at a 90 ◦ angle to each other to create currents in opposite direction. This ensured a thorough circulation of water and removed the settled particles, if any, from the surface of the leaf blades. The filters were checked on a weekly basis to ensure they were not blocked and that there was a strong enough current flowing constantly. Deionized water was added to the main filtration unit, when needed, to maintain the volume and the salinity. The seagrass was cleaned manually once a week, before taking measurements with the PAM, from macro algae which started to grow in the tanks and on the seagrass.

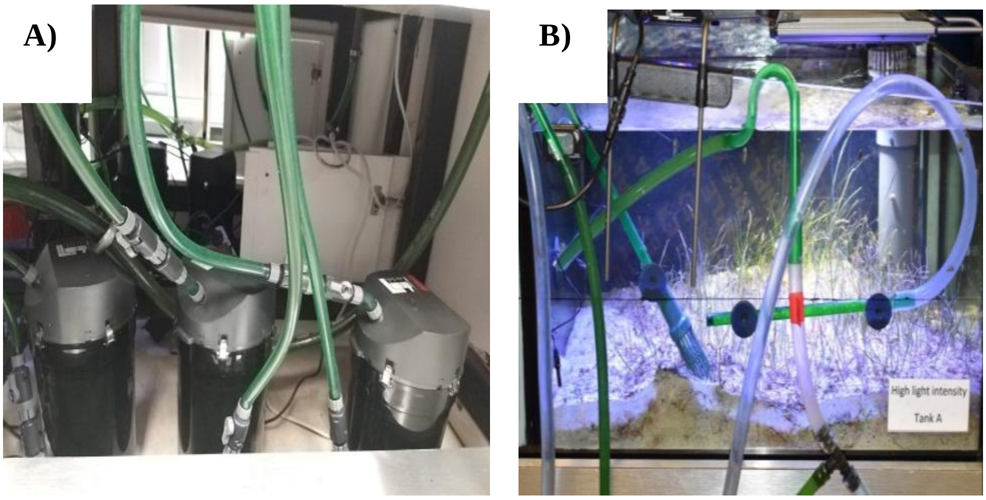


Figure 9: A) The set-up of filtration units B) Set-up of the filtration outlets at 90◦ to each other.

3.5 Experiment to study impact of various light intensity

3.5.1 Experimental set up

In order to test the impact of different light intensities on the growth of seagrass, the tank were divided into three groups, with two tanks in each group. The three groups were labelled as High Intensity, Medium Intensity and Low Intensity, referring to the amount of light provided. Table 1 below shows the different arrangement of LED lights used to obtain the desired light intensities for this experiment.

Table 1: Specifications of light set-ups for the experiment

Light intensity	High	Medium	Low
# of LED lights	3	2	0
Kelvin spectra of lights	• 2x 8000K • 1x 16000K	• 1x 8000K • 1x 16000K	N/A
Color of lights	• Blue • White	• Blue • White	N/A
Intensity of lights (PAR)	452	227	0

Each Tank was provided with its own light system, using a combination of two different LED aquarium lights (Maxspect R420R) as detailed in the Table 1. For the high light intensity, three aquarium lights were placed above the tank, two of which were 60W- 8000K and one of 60W- 16000K. For the medium light intensity there were two lights placed one of 60W-8000K and one of 60W- 16000K. The low light intensity had no lights placed. The lighting system had two light spectra (white, and royal blue). For this experiment both spectra were adjusted to give the highest light intensity possible. To separate the tanks receiving different light intensity, black water proof, thick and rigid plastic partition sheets were placed in between the tanks to ensure no interference between the different light intensity set-ups. This is demonstrated in the Figure 8. The lights were fitted with automated timers to give a photoperiod of 12h light: 12h darkness.

A horizontal line was drawn as shown in the Figure 10 across all tanks representing an eye estimate of the average height of the seagrass in each tank before and at the termination of the light experiment. This line gave an estimate of the changes in seagrass height in different experimental light set-ups.

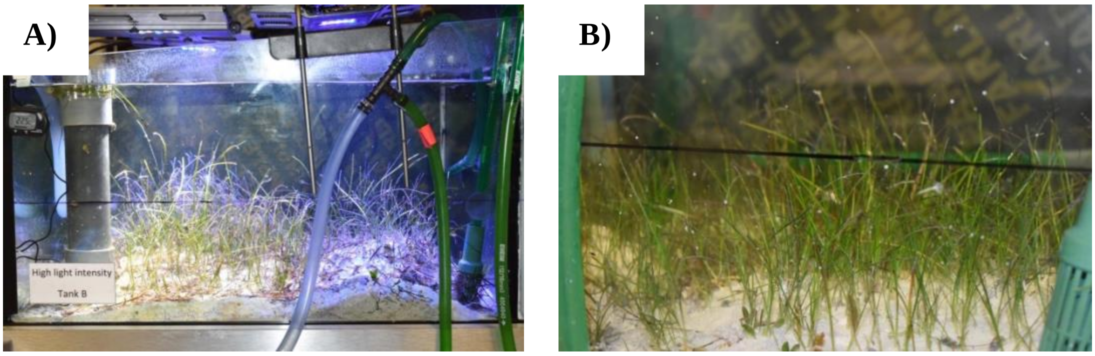


Figure 10: A) The horizontal line drawn of average height of seagrass B) A closer look at the line drawn.

3.5.2 Assessment of photosynthetic activity using the Diving PAM fluorometer

Chlorophyll *a* fluorescence of the seagrass was measured using the diving pulse-amplitude modulated fluorometer (PAM; Walz, Effeltrich©, Germany). This instrument was used to measure maximal quantum yield (YII) of photosystem II after dark adaption for an hour. It has been shown that an hour of darkness is sufficient to reset the photosystem (Salih *et al.*, 2006). Maximum Quantum Yield, is a reliable measure of the potential quantum yield of PS II (Colliers *et al.*, 2016). PAM measurements were taken once a week, for 56 days for each tank. Fifty replicate measurements spanning the entire tank were taken at each time to give the best representation of the fluorescence (Figure

11). PAM measurements were taken early afternoon in a dark environment, with tank and laboratory lights switched off for one hour before the PAM measurement.

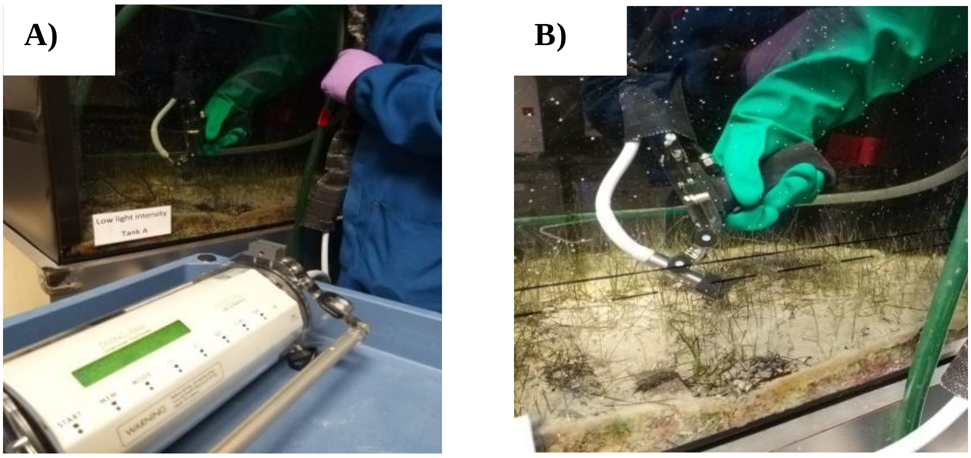


Figure 11: A) demonstrates how the diving PAM was used for measurements. B) A close up view of how measurements were taken.

3.6 Data analysis

Data were analyzed using an analysis of variance (ANOVA) to assess the statistical differences between the seagrass response (i.e., Maximum Quantum Yield) to the different light intensities (treatment) using Excel.

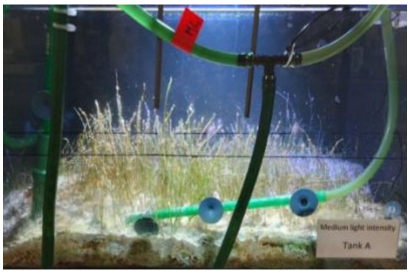
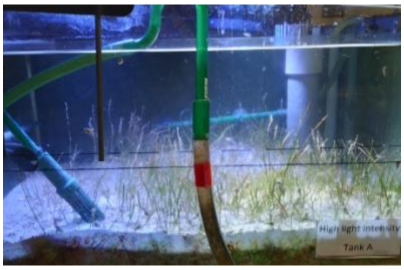
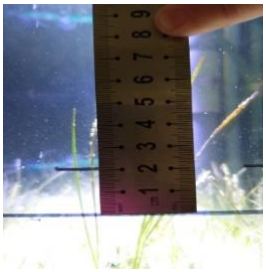
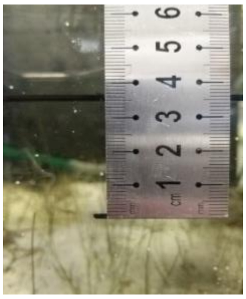
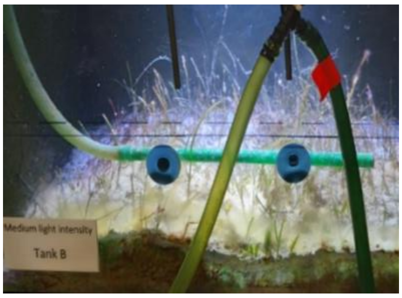
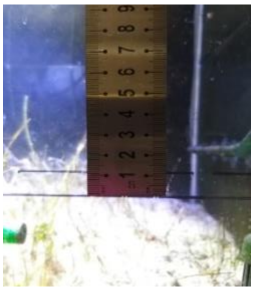
CHAPTER 4: RESULTS

4.1 Water chemistry

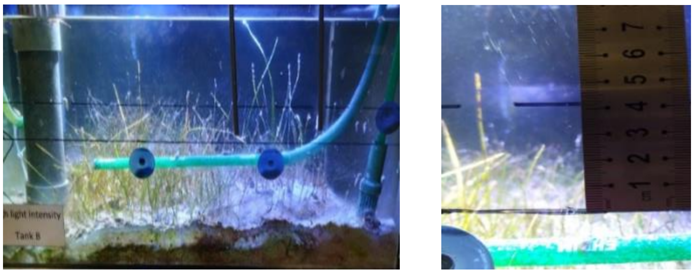
The pH and temperature stayed roughly constant throughout the experiment. The pH was 8.0 ± 0.3 and temperature was 22 ± 2°C. Dissolved oxygen (DO) was 6.14 ± 1.0 mg/L throughout the experiment. Phosphate (PO_4) was within an acceptable value of <0.02 mg/L for all the tanks throughout the experiment. Ammonium (NH_4) was within acceptable value during the experiment and was the same for all the tanks at <0.05 mg/L. Nitrate (NO_3) was maintained at 1 mg/L and was the same for all the tanks throughout the experiment. Nitrites (NO_2) were also constant throughout the experiment and was 0.05 mg/L. TOC level was slightly higher in all the tanks and was 3 ± 1.0 mg/L which, is to be expected due to the vast amount of organisms present in the tanks as well as the amount of seagrass. All monitoring results of water quality parameters and figures are reported in Appendix A.

4.2 Average height of seagrass

Treatment	Average height	
Tank A low		 Decrease in average height of 1.6 cm

Tank A medium		 Increase in average height of 3.0 cm
Tank A high		 Increase in average height of 2.1 cm
Tank B low		 Decrease in average height of 3.6 cm
Tank B medium		 Increase in average height of 1.2 cm

Tank B high



Increase in average height of 4.2 cm

Figure 12: The black lines on the tanks representing the average height of seagrass in different light treatments at the start (solid line) and at the termination (dotted line) of the experiment

Table 2: The difference in average height of the seagrass

Treatment (light intensity)	Initial height (cm)	Final height (cm)	% change
Low (0 PAR)	15.0	12.4	17.3*
Medium (227 PAR)	15.0	17.2	14.0
High (452 PAR)	15.0	18.15	21.0

* This was a reduction in height

All the tanks receiving light showed an increase in the average height of seagrass at the termination of the experiment (dotted line in Figure 12) compared to the initial height (solid line). The highest light intensity (452 PAR) resulted in about 21 % increase in average height of the seagrass compared to the initial height of the seagrass population when they were brought in the lab from the field (Figure 12 & Table 2). This was 7 % more than what was seen in the medium light intensity (227

PAR). In contrast, both replicates of the low light (0 PAR) showed a decline in the average height of the seagrass (Table 2, Figure 13).

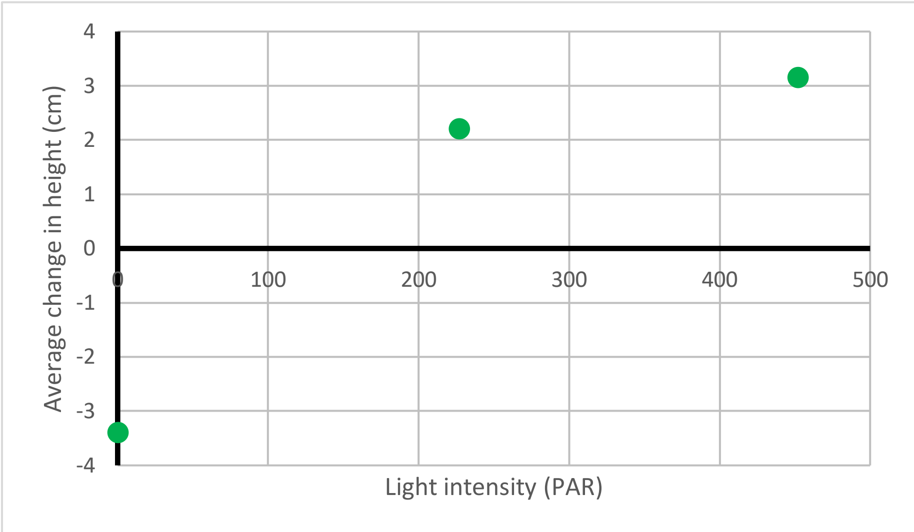
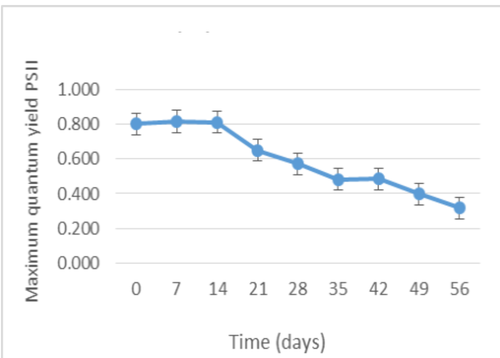
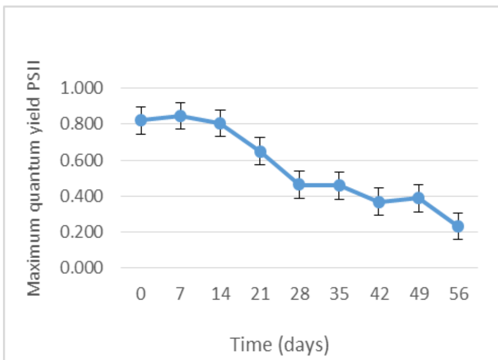


Figure 13: The average increase/decrease in height of seagrass with changing light intensity

4.3 Maximum Quantum Yield (YII)

	Maximum Quantum Yield (II)	
	Tank A	**Tank B**
Low (0 PAR)		

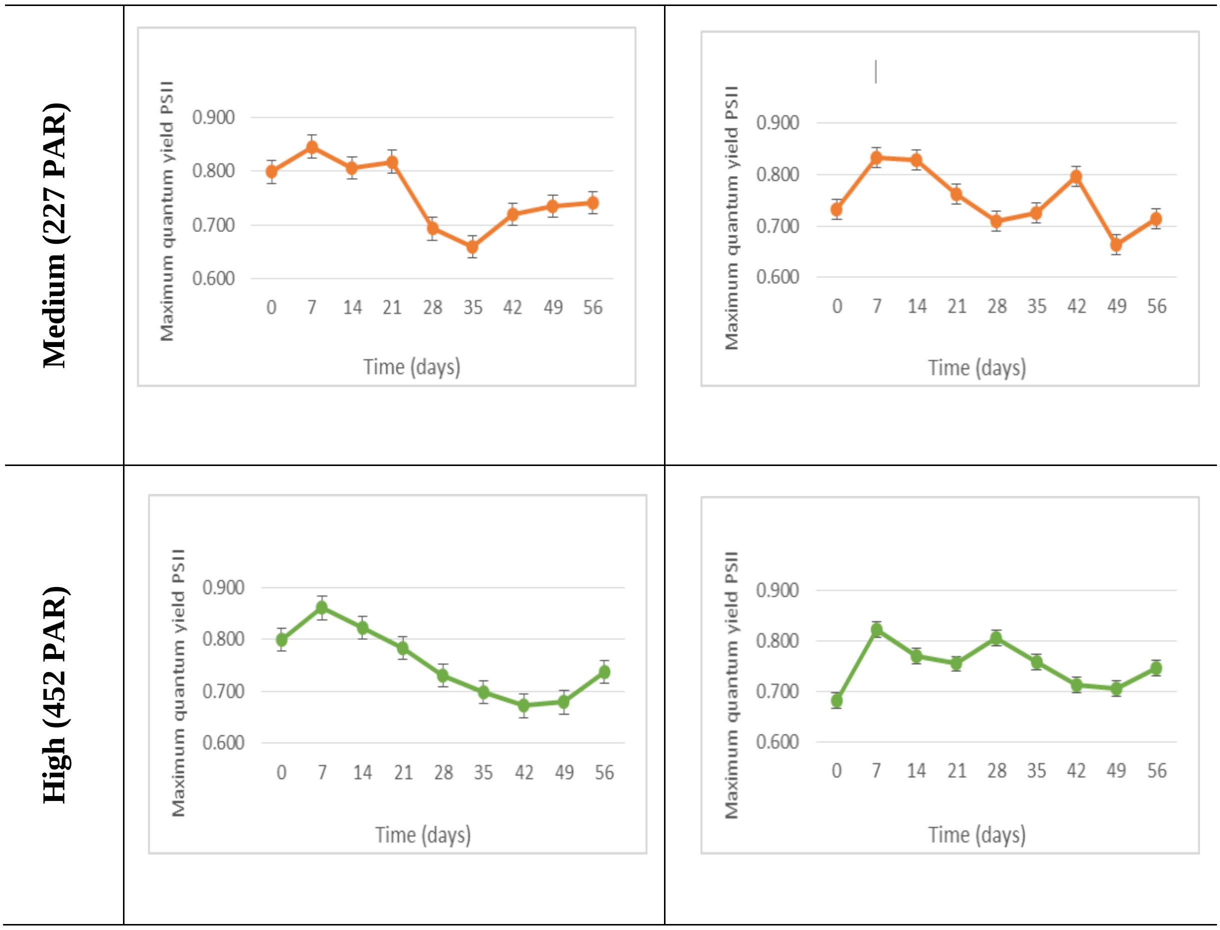


Figure 14: Changes in the photosynthetic activity (Maximum Quantum Yield) with time for replicate A (left panel) and B(right panel) of low, medium and high light treatment. Please note the different scale on the Y-axis

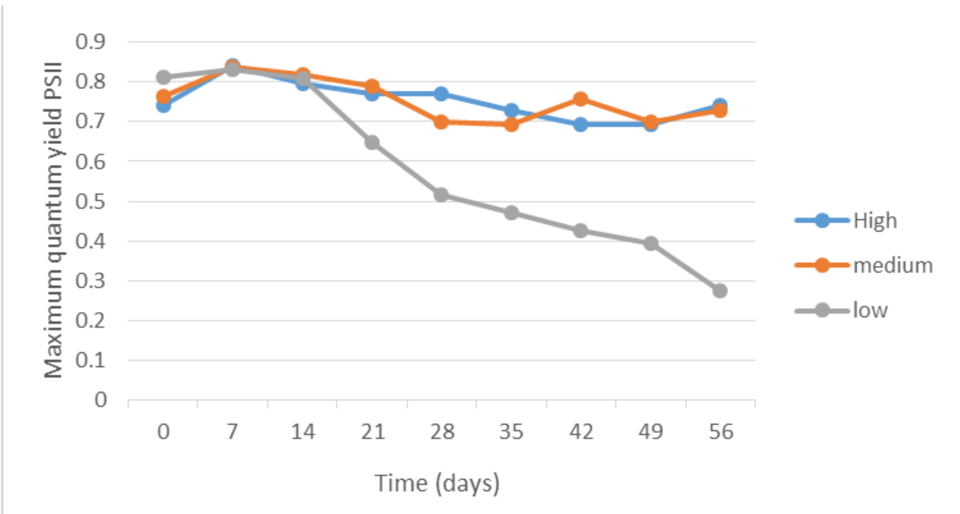


Figure 15. The average YII of Tank A and B measured during the experiment for all three light treatments.

Analysis of Variance (ANOVA) was calculated for both replicates of all light treatments to understand the changes in the measured value of YII over time. The YII of the two replicates (A and B) within any treatment (Low, Medium, High light) did not show any statistically significant difference ($p > 0.05$, Two Way-ANOVA). The whole comprehensive data set analysis showed that there was an influence of light on YII over time. However, when splitting the data set to compare between the different light intensities, the main source of effect was shown when comparing the low light intensity with both medium and high light intensity (ANOVA, Appendix C).

A significant decline in the YII for both tanks receiving low light intensity ($p<0.05$; Appendix C) is very clear in Figure 14. The decline is most apparent between 3-4 weeks (day 28 to 35) of the experiment. In these tanks receiving no light, by the end of the experiment, the health condition measured as YII, declined by 66 % of the initial health condition.

The YII for the medium light intensity went up in the first two weeks for both replicates. However, in the third week (day 21) a decline was observed in this treatment. This was again followed by an increasing trend in YII till the end of the experiment. When exposed to a high light intensity, the YII either maintained around the initial level (Tank B High Light) or declined (Tank A high light). However, these fluctuations in YII in different weeks were not statistically significant ($p > 0.05$, Appendix C) for both the treatments with Medium or High light. Despite the fluctuations recorded in in-between weeks, for all tanks receiving light (Medium and High), the YII at the end of the experiment was similar to YII measured at the initiation of the experiment.

Most importantly, the YII of both replicate tanks receiving no light were significantly lower (0.275) than the YII of both Medium (0.727) and High (0.743) light treatment (p< 0.05, ANOVA, Figure 15). The YII showed a positive correlation to the increase in the light intensity (Figure 16). However, an increase in intensity above 227 PAR did not increase the YII of the seagrass. The very little increase in YII of the High Intensity was not significantly higher than the YII recorded in Medium Intensity (p>0.05, ANOVA).

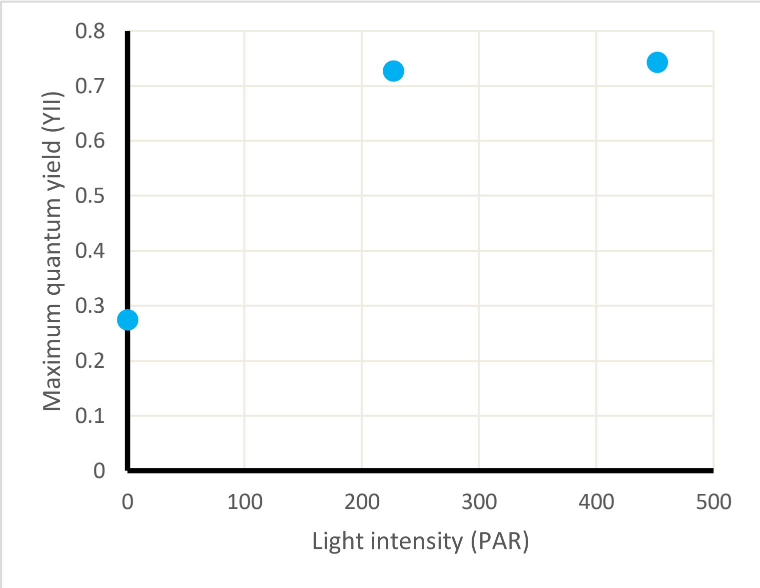


Figure 16. The average maximum quantum yield (YII) in relation to the intensity of light measured in PAR.

CHAPTER 5: DISCUSSION

The Arabian Gulf waters present a very challenging environment to its marine organisms due to very high temperature and salinity conditions that can go up to 36 °C and 80 PSU (Naser, 2014). Additionally, the rapid expansion in development around the Gulf (Naser, 2014) can potentially cause a threat to its marine biota already working at its extreme tolerance limits. Seagrass plays a vital role in the marine environment as discussed earlier and therefore studying them is crucial in order to understand how they could be impacted by anthropogenic activities in and around the Arabian Gulf.

Despite the important role of the seagrass in the marine food web, not much is known about this species and its ecological requirements. Due to a lack of knowledge about the seagrass distribution, requirements for its optimum growth, natural variability and risk assessments, no management plan has been implemented to protect the seagrass in their natural habitat globally (Long & Thom, 2001). This is primarily because it is hard to maintain and grow seagrass under laboratory conditions. Since it is also laborious to study seagrass in the field, it is important to establish a protocol to maintain seagrass in the lab to be able to study factors that could affect its growth and functioning. The results obtained from this study clearly establish that it is possible to maintain and grow seagrass under controlled laboratory conditions. This is an important finding as it opens the possibility of studying impacts of various stressors, such as turbidity, change in temperature, carbon capture etc. on the performance of the seagrass (Duarte *et al.*, 2013). A biological filtration unit with recirculating water to maintain water quality, use of pumps to generate current, and a basic LED aquarium light providing a light intensity at 227 PAR (the medium light treatment in this study) was sufficient enough to maintain the seagrass in laboratory tanks. The temperature was maintained at 22 ºC and salinity at 40 psu.

Seagrass has been reported to show a linear increase in growth with the increase in light intensity (Short *et al.,* 1995). In the present study also, the average gain in height of the seagrass showed a positive correlation with the increase in light. However, an increase in light beyond 227 PAR did not increase the photosynthetic performance. Light intensity which plays a critical role in the photosynthesis and productivity of the seagrass can determine the species distribution and proliferation in the marine environment (Hanelt, 1992, Masini *et al.,* 1995). The vast system of roots and rhizomes of the seagrass necessitates a high demand of light with at least 20 - 30 % of surface-incident sunlight for optimal production (Duarte, 1991; Fourqurean *et al.,* 2003). This is higher than other marine plants. In the Qatari coast, especially, in the mangrove areas, this requirement could possibly be even higher as seagrass may have a high photosynthetic demand to survive in anoxic sediments.

One of the greatest stressors to seagrass in the Arabian Gulf and in particular Qatar is the reduction of light that reaches the seagrass due to coastal development, dredging and anthropogenic activities increasing the suspended particle in the water which settles on the leave blades (Erftemeijer *et al.,* 2006). This increases the turbidity causing less light to reach the seagrass. This in turn will affect their photosynthetic ability and may cause a serious decline in seagrass biomass. In the present study this is clearly shown in the treatments that received no light at all for the entire duration of the experiment. In low light conditions, the seagrass population could maintain its photosynthetic activity as measured by PAM for about three weeks. After this period, their growth shows that they were very severely affected. Their YII was drastically (66%) lower than the other tanks receiving light. The seagrass distribution became scattered in the tanks. This points to the negative impact of suspended particles and turbidity in an aquatic ecosystem. Any factor, like turbidity, or settling macroalgae, that reduces the light reaching the seagrass, can affect its photosynthetic performance and hence reduce primary productivity of the ecosystem. This is indicated in this study in the tanks that received light. To elaborate, the fluctuation in the YII measurements during the experiment in tanks with light could be related to an extensive growth of several macroalgal species on the seagrass which reduced the light reaching the leaf blades (Appendix D). The macroalgae frequently covered the seagrass epidermis which is the site for photosynthetic activity. Although these sea grass leaf blades were cleaned to get rid of the macroalgae immediately before conducting the PAM measurements, their photosynthetic ability was negatively affected and gave low YII measurements in the study. Interestingly, after the removal of the macroalgae from the leaves, they recovered their YII values and their health consequently improved and reached the initial YII levels. A similar observation by Paramasivam *et al.,* (2015) suggests that algal blooms can limit the amount of light reaching the leaf blades influencing their distribution depth and overall growth and can cause a severe reduction in seagrass abundance.

Several studies conducted on different species of seagrass (Addicott & Lyon, 1973; Backman & Barilotti, 1976; Bulthuis, 1983) have reported a rapid decline in shoot density as well as in the formation of leaf clusters due to a reduction in light. Although the shoot density was not evaluated in this study, there was a high loss of leaves in the tanks receiving no light pointing to a loss of productivity in the absence of light.

This study is a first step to demonstrate how the minimum or threshold light requirement for optimum growth of sea grass can be derived experimentally under controlled laboratory conditions. However, it would be important to conduct in situ field studies to corroborate the laboratory findings in the natural situation. The incoming irradiance, seasonal temperature changes can affect the abundance and biomass production and these need to be experimentally evaluated to derive a threshold light requirement for this species.

CHAPTER 6: CONCLUSION

The seagrass in the Arabian Gulf coast is crucial for the survival of several marine species including some endangered ones, like Dugong and Green sea turtles (Preen, 2004). In addition to the inherent challenges in this region, like high temperature and salinity, the ecosystem is facing the ever-increasing anthropogenic influences that has the potential to hamper the proper functioning of the marine ecosystem (Sheppard, 1993). These can alter the amount of light reaching the seagrass and affect their primary production and biomass as they depend on light for photosynthesis. This study emphasizes this important role of light in altering the health of the seagrass population. The seagrass population showed a drastic fall in health in an extreme situation with no or barely any light. The present study also clearly shows that it is possible to maintain seagrass population under controlled laboratory conditions where seagrass not only could maintain their health, but also grew appreciably when provided with light. Establishing a protocol for sustaining *Halodule uninervis* seagrass in the laboratory is a very important contribution as it will make it feasible to design experiments to understand this seagrass species basic requirements and relation with abiotic and biotic factors. It will be important to conduct further studies to ascertain the role of other environmental factors, like temperature, salinity, toxicants, presence of competitive species to determine the biological threshold for optimum sustenance of seagrass and set environmental guidelines for conserving, restoring or relocating this crucial component of the marine ecosystem.

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APPENDIX

APPENDIX (A): WATER QUALITY PARAMENTERS AND FIGURES

Water quality parameters:

Temperature (◦C)

Light intensity:	**High**		**Medium**		**Low**	
Date	**Tank A**	**Tank B**	**Tank A**	**Tank B**	**Tank A**	**Tank B**
16-Jan	22.7	22.6	23.2	22.6	22.7	22.6
23-Jan	22.8	22.8	23.0	22.8	22.8	22.8
30-Jan	22.5	22.6	22.8	22.6	22.7	22.6
6-Feb	22.8	22.8	22.9	22.7	22.8	22.8
14-Feb	23.0	23.2	23.0	23.2	23.1	23.2
21-Feb	23.2	22.9	23.2	23.0	23.2	23.0
27-Feb	22.9	22.8	22.9	22.8	23.0	22.8
6-Mar	22.7	23.1	23.1	23.1	22.7	23.1
13-Mar	22.8	22.9	23.5	23.1	22.8	22.9

Salinity (psu)

Light intensity:	**High**		**Medium**		**Low**	
Date	**Tank A**	**Tank B**	**Tank A**	**Tank B**	**Tank A**	**Tank B**
16-Jan	40	40	40	40	40	40
23-Jan	40	40	40	40	40	40
30-Jan	40	40	40	40	40	40
6-Feb	40	40	40	40	40	40
14-Feb	40	40	40	40	40	40
21-Feb	40	40	40	40	40	40
27-Feb	40	40	40	40	40	40
6-Mar	40	40	40	40	40	40
13-Mar	40	40	40	40	40	40

Light intensity (PAR)

Light intensity:	**High**		**Medium**		**Low**	
Date	**Tank A**	**Tank B**	**Tank A**	**Tank B**	**Tank A**	**Tank B**
16-Jan	452	451	229	225	2	2
23-Jan	451	453	228	229	1	1
30-Jan	453	453	229	228	0	0
6-Feb	451	452	227	227	0	1
14-Feb	454	454	228	225	1	0

21-Feb	453	453	226	229	0	0
27-Feb	453	455	228	228	0	0
6-Mar	452	452	227	227	1	1
13-Mar	455	453	229	226	2	0
average	452.67	452.89	227.89	227.11	0.78	0.56

pH

Light intensity:	**High**		**Medium**		**Low**	
Date	**Tank A**	**Tank B**	**Tank A**	**Tank B**	**Tank A**	**Tank B**
16-Jan	8.09	8.08	8.09	8.10	8.07	8.13
23-Jan	8.09	8.09	8.09	8.10	8.08	8.10
30-Jan	8.08	8.09	8.08	8.09	8.08	8.11
6-Feb	8.09	8.08	8.09	8.09	8.09	8.15
14-Feb	8.09	8.08	8.09	8.08	8.09	8.13
21-Feb	8.08	8.09	8.08	8.09	8.08	8.10
27-Feb	8.08	8.08	8.08	8.07	8.08	8.07
6-Mar	8.09	8.08	8.09	8.06	8.09	8.13
13-Mar	8.09	8.08	8.09	8.09	8.09	8.11

DO (mg/L)

Light intensity:	**High**		**Medium**		**Low**	
Date	**Tank A**	**Tank B**	**Tank A**	**Tank B**	**Tank A**	**Tank B**
16-Jan	7.67	8.17	7.44	7.49	6.51	6.65
23-Jan	7.52	7.75	7.24	7.25	6.23	6.35
30-Jan	7.34	7.93	6.44	7.00	6.76	6.87
6-Feb	6.80	7.62	6.94	6.48	6.82	6.43
14-Feb	7.20	6.95	7.01	6.49	6.21	6.98
21-Feb	7.15	6.98	6.85	7.11	6.84	6.67
27-Feb	7.03	7.41	6.23	7.35	6.23	6.61
6-Mar	6.83	7.65	7.23	7.30	6.94	6.14
13-Mar	6.71	6.98	6.95	6.98	6.24	6.55

NO_2 (mg/L)

Light intensity:	**High**		**Medium**		**Low**	
Date	**Tank A**	**Tank B**	**Tank A**	**Tank B**	**Tank A**	**Tank B**
16-Jan	0.05	0.05	0.05	0.05	0.05	0.05

23-Jan	0.05	0.05	0.05	0.05	0.05	0.05
30-Jan	0.05	0.05	0.05	0.05	0.05	0.05
6-Feb	0.05	0.05	0.05	0.05	0.05	0.05
14-Feb	0.05	0.05	0.05	0.05	0.05	0.05
21-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	0.05	0.05	0.05	0.05	0.05	0.05
13-Mar	0.05	0.05	0.05	0.05	0.05	0.05

NO_3 (mg/L)

Light intensity:	**High**		**Medium**		**Low**	
Date	**Tank A**	**Tank B**	**Tank A**	**Tank B**	**Tank A**	**Tank B**
16-Jan	1	1	1	1	1	1
23-Jan	1	1	1	1	1	1
30-Jan	1	1	1	1	1	1
6-Feb	1	1	1	1	1	1
14-Feb	1	1	1	1	1	1
21-Feb	1	1	1	1	1	1
27-Feb	1	1	1	1	1	1
6-Mar	1	1	1	1	1	1
13-Mar	1	1	1	1	1	1

NH_4

Light intensity:	**High**		**Medium**		**Low**	
Date	**Tank A**	**Tank B**	**Tank A**	**Tank B**	**Tank A**	**Tank B**
16-Jan	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
23-Jan	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
30-Jan	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
6-Feb	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
14-Feb	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
21-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	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
13-Mar	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

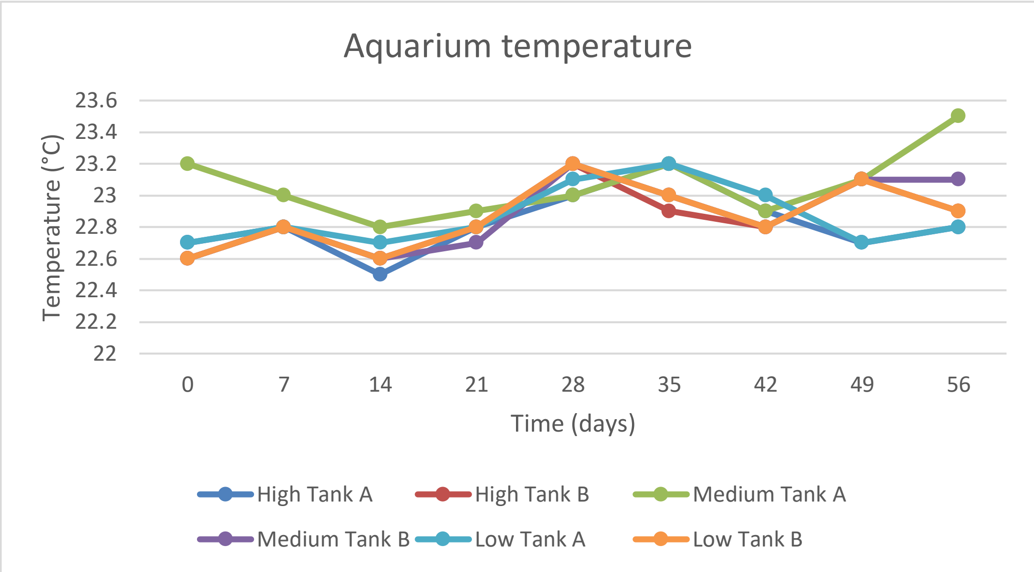
PO_4

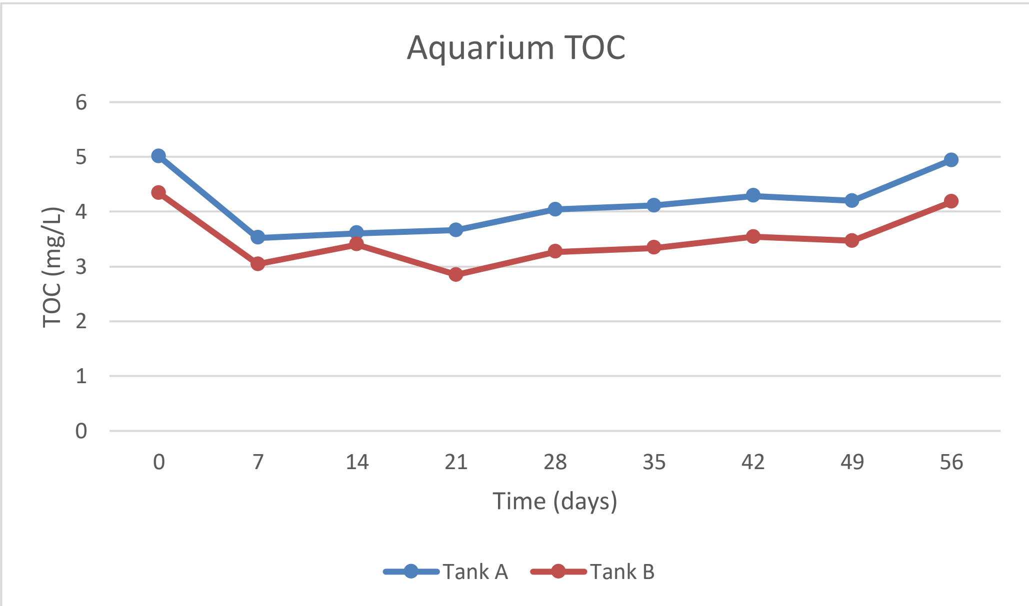
Light intensity:	High		Medium		Low	
Date	**Tank A**	**Tank B**	**Tank A**	**Tank B**	**Tank A**	**Tank B**
16-Jan	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
23-Jan	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
30-Jan	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
6-Feb	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
14-Feb	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
21-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
13-Mar	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02

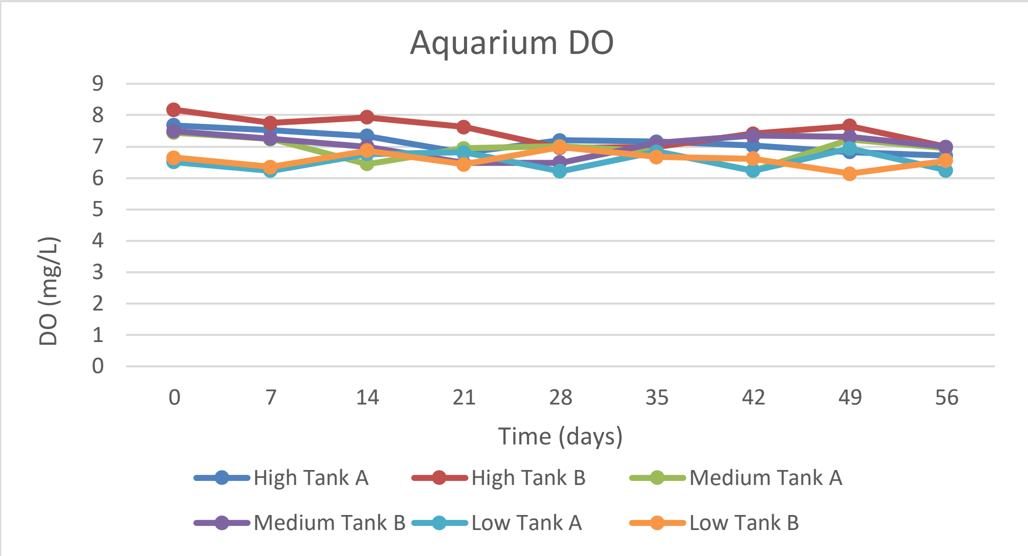
TOC (mg/L)

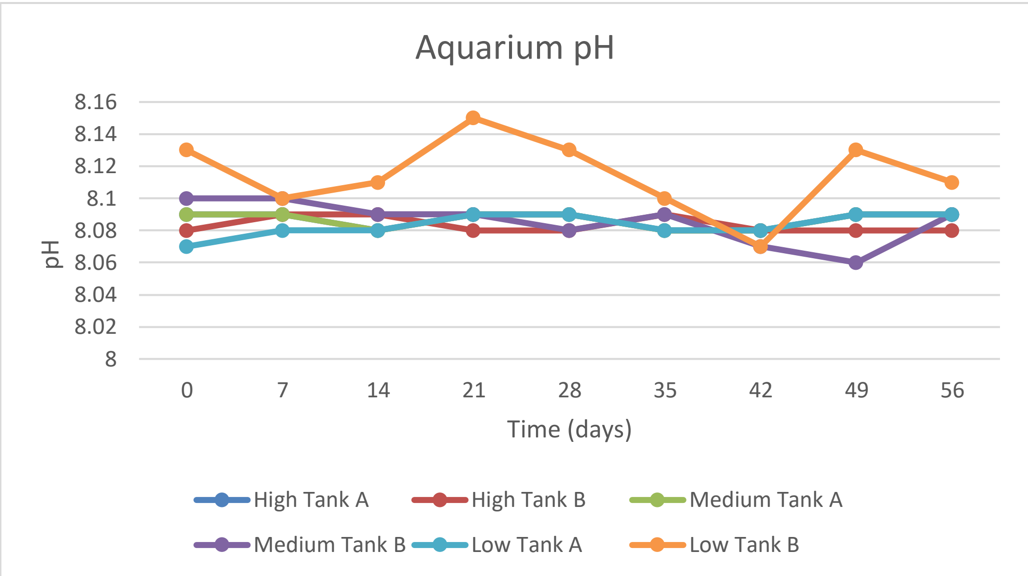
Date	Tank A	Tank B
16-Jan	5.01	4.34
23-Jan	3.52	3.04
30-Jan	3.61	3.4
6-Feb	3.66	2.85
14-Feb	4.04	3.27
21-Feb	4.11	3.34
27-Feb	4.29	3.54
6-Mar	4.2	3.47
13-Mar	4.94	4.18

Water quality figures:






Aquarium DO
DO (mg/L)
9
8
7
6
5
4
3
2
1
0
0
7
14
21
28
35
42
49
56
Time (days)
High Tank A
High Tank B
Medium Tank A
Medium Tank B
Low Tank A
Low Tank B


Aquarium pH
pH
8.16
8.14
8.12
8.1
8.08
8.06
8.04
8.02
8
0
7
14
21
28
35
42
49
56
Time (days)
High Tank A
High Tank B
Medium Tank A
Medium Tank B
Low Tank A
Low Tank B

APPENDIX (B): YIELD READINGS

16 January 2019						
Replicate	**Tank A**			**Tank B**		
Number	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**
1	0.933	0.912	0.954	0.708	0.959	0.843
2	0.944	0.500	0.858	0.500	0.500	0.575
3	0.725	0.692	0.910	0.833	0.222	0.465
4	0.897	0.100	0.907	0.285	0.444	0.819
5	0.600	0.515	0.769	0.947	0.750	0.100
6	0.535	0.625	0.428	0.823	0.848	0.100
7	0.535	0.333	0.853	0.669	0.854	0.679
8	0.518	0.909	0.362	0.787	0.409	0.962
9	0.796	0.968	0.920	0.943	0.939	0.647
10	0.912	0.678	0.947	0.148	0.959	0.881
11	0.100	0.626	0.956	0.707	0.839	0.901
12	0.829	0.640	0.798	0.853	0.592	0.987
13	0.917	0.989	0.915	0.657	0.862	0.100
14	0.897	0.100	0.833	0.296	0.765	0.993
15	0.627	0.281	0.800	0.897	0.833	0.935
16	0.800	0.100	0.892	0.756	0.957	0.440
17	0.904	0.526	0.884	0.760	0.612	0.810
18	0.952	0.368	0.615	0.816	0.935	0.100
19	0.970	0.547	0.857	0.859	0.684	0.760
20	0.717	0.921	0.819	0.312	0.956	0.911
21	0.913	0.981	0.639	0.890	0.576	0.827
22	0.802	0.791	0.907	0.920	0.196	0.753
23	0.100	0.851	0.351	0.928	0.787	0.994
24	0.849	0.870	0.931	0.932	0.793	0.902
25	0.941	0.925	0.890	0.846	0.827	0.952
26	0.922	0.100	0.612	0.206	0.857	0.912
27	0.750	0.722	0.874	0.946	0.901	0.567
28	0.933	0.987	0.653	0.995	0.947	0.964
29	0.972	0.666	0.929	0.838	0.100	0.721
30	0.966	0.854	0.480	0.877	0.775	0.874
31	0.988	0.928	0.949	0.944	0.936	0.881
32	0.625	0.807	0.823	0.604	0.379	0.885
33	0.944	0.962	0.875	0.791	0.888	0.891
34	0.975	0.704	0.250	0.797	0.410	0.809
35	0.384	0.813	0.956	0.384	0.500	0.944
36	0.500	0.951	0.100	0.218	0.966	0.986
37	0.902	0.920	0.100	0.543	0.863	0.914
38	0.533	0.966	0.925	0.800	0.292	0.920

39	0.500	0.909	0.962	0.472	0.903	0.673
40	0.868	0.764	0.666	0.375	0.961	0.725
41	0.842	0.380	0.992	0.520	0.731	0.421
42	0.714	0.965	0.382	0.454	0.765	0.938
43	0.853	0.994	0.963	0.634	0.823	0.586
44	0.881	0.847	0.919	0.600	0.343	0.937
45	0.522	0.990	0.976	0.697	0.762	0.633
46	0.822	0.666	0.828	0.490	0.652	0.881
47	0.797	0.941	0.786	0.860	0.776	0.840
48	0.612	0.857	0.986	0.899	0.230	0.451
49	0.869	0.902	0.369	0.526	0.879	0.864
50	0.827	0.965	0.960	0.517	0.958	0.812
Average	**0.76428**	**0.72616**	**0.7662**	**0.68118**	**0.7139**	**0.7493**

23 January 2019						
Replicate	**Tank A**			**Tank B**		
Number	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**
1	0.100	0.529	0.488	0.900	0.935	0.821
2	0.647	0.877	0.846	0.868	1.000	0.600
3	0.864	0.949	0.511	0.938	0.878	0.562
4	0.970	0.757	0.900	0.864	0.627	0.673
5	0.625	0.668	0.791	0.977	0.781	1.000
6	0.733	1.000	0.469	0.901	0.818	0.887
7	0.985	0.843	0.909	0.958	0.852	0.941
8	1.000	0.997	0.961	0.583	0.516	0.792
9	0.785	0.980	1.000	0.610	0.804	0.775
10	0.875	0.966	0.922	0.985	0.861	0.960
11	0.938	0.658	0.976	0.699	0.819	0.770
12	1.000	0.750	1.000	0.470	0.964	0.750
13	0.761	0.970	0.625	0.777	0.416	0.930
14	0.100	1.000	1.000	0.955	0.808	0.939
15	0.967	1.000	0.875	0.601	0.869	0.974
16	1.000	0.946	0.948	0.921	0.750	0.975
17	1.000	0.684	0.846	1.000	0.833	0.719
18	0.966	0.678	0.769	0.886	0.963	0.969
19	0.961	1.000	0.878	1.000	0.785	0.714
20	0.823	0.632	0.918	0.937	0.769	0.655
21	0.600	0.952	0.461	0.977	1.000	0.968
22	0.600	0.844	1.000	0.933	0.777	0.978
23	0.844	0.882	0.913	0.960	0.910	1.000
24	0.990	0.857	0.347	0.776	0.953	1.000
25	0.868	0.946	0.357	0.641	0.781	0.931

26	1.000	0.517	0.857	0.909	0.851	0.785
27	1.000	0.755	1.000	0.900	0.978	0.710
28	0.875	0.815	0.423	0.912	1.000	0.994
29	0.863	0.922	1.000	0.627	1.000	0.608
30	0.774	0.836	0.578	0.859	0.466	0.563
31	0.617	0.888	1.000	0.906	0.986	0.972
32	0.908	0.734	0.783	0.934	1.000	0.719
33	0.926	0.961	0.964	0.578	1.000	0.891
34	0.600	0.936	0.666	0.788	0.605	0.903
35	0.803	1.000	0.826	0.642	0.944	0.846
36	1.000	1.000	0.949	0.629	0.469	0.950
37	0.894	1.000	1.000	0.782	0.625	0.695
38	0.760	0.575	0.923	0.940	0.990	0.921
39	0.926	0.666	0.970	0.972	0.743	0.959
40	0.922	0.833	0.932	0.846	0.877	0.869
41	0.871	1.000	0.561	0.571	0.923	1.000
42	1.000	0.703	0.901	0.555	1.000	1.000
43	0.947	0.816	0.850	0.787	0.968	0.792
44	0.963	0.900	0.970	0.750	1.000	0.777
45	0.714	0.826	0.903	1.000	0.809	1.000
46	0.833	0.870	0.647	0.933	1.000	0.804
47	0.612	0.736	0.985	0.863	0.968	0.833
48	0.675	0.705	0.602	0.633	0.558	0.921
49	0.782	1.000	0.936	0.904	0.517	0.600
50	0.976	0.914	0.950	0.818	0.840	0.881
Average	**0.82486**	**0.84546**	**0.8177**	**0.8231**	**0.83172**	**0.84552**

30 January 2019						
Replicate	**Tank A**			**Tank B**		
Number	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**
1	0.858	0.956	0.957	0.881	0.964	0.940
2	0.883	0.931	0.898	0.860	0.924	0.456
3	1.000	0.985	1.000	0.674	0.857	0.891
4	1.000	1.000	0.500	0.371	0.831	0.863
5	0.459	0.650	0.784	0.599	0.588	0.529
6	0.974	0.968	0.875	0.864	0.972	0.768
7	0.775	0.850	1.000	0.825	0.632	0.619
8	0.961	0.984	0.937	0.589	1.000	0.854
9	0.400	1.000	0.869	0.525	1.000	0.925
10	0.880	0.644	1.000	0.450	0.863	0.692
11	0.978	0.594	0.800	0.697	0.562	1.000
12	0.553	0.990	0.980	0.631	0.394	0.988
13	0.913	0.705	0.125	0.929	0.986	1.000

14	0.264	0.791	0.100	0.500	0.967	1.000
15	0.913	1.000	0.938	1.000	0.903	0.820
16	0.961	0.520	0.800	0.269	0.589	0.778
17	1.000	0.466	0.763	0.815	0.770	0.133
18	1.000	0.333	1.000	0.944	0.943	0.938
19	0.957	1.000	0.363	0.984	0.862	0.911
20	0.800	0.956	0.981	0.974	0.600	0.938
21	0.760	0.952	1.000	1.000	0.734	0.500
22	0.666	0.967	0.797	0.959	0.645	1.000
23	0.615	0.346	0.944	0.842	0.682	0.773
24	0.950	1.000	0.500	0.646	1.000	0.894
25	0.923	0.646	0.655	0.778	1.000	0.196
26	0.711	0.559	0.960	0.729	1.000	0.718
27	0.858	0.707	0.947	0.953	0.961	0.809
28	0.983	0.531	0.200	0.800	0.951	0.507
29	1.000	0.948	1.000	0.995	0.850	0.931
30	0.954	0.314	1.000	0.620	0.717	1.000
31	0.965	0.855	0.929	0.608	0.904	0.605
32	0.689	0.948	0.869	0.902	0.922	1.000
33	0.918	0.969	0.823	0.763	0.890	0.642
34	0.979	0.711	0.972	0.958	0.935	1.000
35	0.974	0.680	1.000	0.769	0.600	1.000
36	0.580	0.981	0.894	0.749	0.848	0.773
37	0.890	0.611	0.673	0.852	0.680	0.822
38	0.725	0.962	0.400	0.693	0.774	1.000
39	0.970	0.666	0.696	0.670	0.870	0.866
40	0.894	0.923	0.951	0.500	0.966	0.653
41	0.913	0.965	0.666	0.618	0.833	0.858
42	0.904	1.000	1.000	0.793	0.875	0.691
43	0.863	1.000	0.916	0.666	0.100	0.794
44	0.917	0.962	0.591	1.000	0.840	1.000
45	0.935	0.833	0.992	0.966	1.000	1.000
46	0.837	0.478	0.692	0.880	0.600	0.978
47	0.644	0.854	0.909	0.851	0.936	0.695
48	0.704	1.000	0.588	0.859	0.609	0.823
49	0.370	1.000	0.808	0.903	1.000	1.000
50	0.459	0.600	0.742	0.857	0.627	0.694
Average	0.82158	0.80582	0.7956	0.771	0.81112	0.8053

6 February 2019						
Replicate	**Tank A**			**Tank B**		
Number	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**
1	1.000	1.000	1.000	1.000	0.832	0.000
2	1.000	0.875	0.000	0.967	0.614	0.641

3	0.886	0.825	1.000	0.965	0.735	0.995
4	0.840	0.967	0.000	0.355	0.655	0.000
5	1.000	0.994	0.958	0.476	0.963	0.954
6	1.000	0.745	0.800	0.984	0.765	0.000
7	0.987	0.712	0.583	0.791	0.466	0.846
8	1.000	0.809	0.000	0.906	0.480	0.000
9	0.555	0.958	0.366	0.875	0.885	0.888
10	0.690	0.796	0.968	0.810	0.913	0.000
11	0.800	0.925	0.400	0.971	0.625	0.913
12	0.555	0.333	0.500	0.915	0.689	1.000
13	0.025	0.774	0.307	0.763	0.990	1.000
14	0.975	0.954	0.810	0.961	0.901	0.500
15	1.000	0.884	0.867	0.909	1.000	0.911
16	0.450	0.688	1.000	0.683	0.976	0.813
17	0.924	0.312	0.000	0.926	0.812	0.000
18	0.823	0.975	0.533	0.284	0.888	0.790
19	1.000	0.957	0.000	0.886	0.900	0.000
20	1.000	0.800	0.666	0.487	0.755	0.941
21	0.958	0.833	0.934	0.833	0.797	0.227
22	0.916	0.656	0.437	0.884	0.875	0.807
23	0.528	0.978	0.823	0.428	0.997	0.977
24	0.917	0.382	0.819	0.200	0.472	0.800
25	0.926	0.754	0.812	0.989	0.948	1.000
26	0.925	0.875	1.000	0.250	0.403	0.000
27	0.989	0.821	1.000	0.834	0.890	0.937
28	0.676	1.000	0.857	0.821	0.944	0.000
29	0.794	1.000	1.000	0.869	0.333	1.000
30	0.300	0.626	0.844	0.681	0.960	0.601
31	0.823	0.946	0.513	0.436	0.388	0.000
32	0.818	0.963	0.460	0.604	0.884	0.810
33	0.625	0.546	0.886	0.740	0.666	0.841
34	0.829	0.750	0.776	0.877	0.545	0.200
35	1.000	0.900	1.000	0.818	0.951	0.426
36	1.000	0.432	0.444	0.867	0.883	0.848
37	1.000	0.971	0.846	0.892	0.333	1.000
38	1.000	0.846	0.824	0.518	0.497	0.872
39	0.333	0.827	0.832	0.837	0.466	0.890
40	0.333	0.782	0.655	0.789	0.557	0.950
41	0.634	1.000	0.931	0.762	0.772	0.991
42	0.948	1.000	0.863	0.791	0.939	0.545
43	0.574	1.000	0.666	0.773	0.988	0.802
44	0.932	1.000	0.689	0.875	1.000	0.991
45	0.768	0.906	0.024	0.617	0.702	0.824
46	0.289	1.000	0.382	0.947	1.000	1.000

47	0.898	0.611	0.989	0.566	0.500	0.833
48	0.924	0.666	0.600	0.531	0.666	0.163
49	0.148	0.714	0.782	0.826	0.861	0.889
50	0.835	0.785	0.000	1.000	1.000	1.000
Average	**0.783**	**0.81706**	**0.64892**	**0.75538**	**0.76122**	**0.64832**

13 February 2019						
Replicate	**Tank A**			**Tank B**		
Number	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**
1	1.000	0.939	0.000	0.343	0.697	1.000
2	0.829	0.913	0.000	0.926	0.747	0.000
3	0.687	0.572	0.875	0.381	0.795	0.666
4	0.647	0.538	0.990	0.965	0.972	0.970
5	0.923	0.288	0.000	0.928	0.974	1.000
6	0.571	0.890	0.854	0.993	0.454	0.000
7	0.242	0.888	0.000	0.909	1.000	0.545
8	0.897	0.184	0.911	0.944	0.428	0.000
9	0.913	0.929	0.000	0.965	0.888	1.000
10	0.833	0.696	0.967	0.166	0.838	0.000
11	0.925	0.945	0.529	0.846	0.961	0.000
12	0.419	0.782	0.785	0.940	0.428	1.000
13	0.343	0.241	0.913	0.983	0.705	0.000
14	0.785	0.481	0.890	0.680	0.979	0.703
15	0.856	0.720	0.866	0.885	0.684	0.823
16	0.849	0.712	0.000	0.914	0.241	0.000
17	0.354	0.900	0.100	0.573	0.473	0.000
18	0.854	0.622	0.000	0.913	0.675	0.000
19	0.865	0.907	0.000	1.000	0.413	0.000
20	0.892	0.881	0.666	0.935	0.523	1.000
21	0.793	0.367	0.906	0.615	0.882	0.000
22	0.611	0.700	0.100	0.941	0.680	1.000
23	0.648	0.782	0.869	0.220	0.194	0.000
24	0.530	0.109	0.600	0.754	1.000	0.000
25	0.989	0.847	0.100	0.892	0.841	1.000
26	0.809	0.745	0.846	0.745	0.465	0.000
27	0.233	0.455	0.100	0.822	0.335	0.898
28	0.787	0.243	0.000	0.868	0.925	0.882
29	0.913	0.912	0.896	0.838	0.477	0.000
30	0.206	0.883	0.608	0.669	0.970	0.655
31	0.989	0.704	0.946	0.920	0.240	0.448
32	0.688	0.841	0.000	0.952	0.784	0.940
33	0.116	0.858	0.000	0.972	0.571	1.000
34	0.566	0.701	0.933	0.428	0.545	0.000

35	0.773	0.460	0.500	0.783	0.983	1.000
36	0.738	0.963	0.000	0.540	0.984	0.000
37	0.467	0.947	0.837	0.851	0.100	1.000
38	0.749	1.000	0.977	0.874	0.384	0.000
39	0.979	0.166	0.000	0.915	1.000	0.894
40	0.707	0.500	0.898	0.882	0.845	0.840
41	0.960	0.803	0.909	0.850	0.950	0.000
42	1.000	0.911	0.000	0.500	0.708	0.901
43	0.863	0.181	0.960	0.919	0.847	0.000
44	0.906	0.727	0.073	0.944	0.673	0.958
45	0.402	0.482	0.516	1.000	0.652	0.959
46	0.938	0.812	0.407	0.902	0.961	1.000
47	0.739	0.902	0.538	0.750	0.745	0.000
48	0.903	0.886	0.752	0.923	0.885	0.000
49	0.840	0.863	0.409	0.866	0.973	0.000
50	1.000	0.873	1.000	1.000	0.987	0.000
Average	**0.73052**	**0.69302**	**0.5005**	**0.8064**	**0.70922**	**0.46164**

20 February 2019						
Replicate	**Tank A**			**Tank B**		
Number	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**
1	0.937	0.746	0.521	0.607	0.965	0.000
2	0.878	0.626	0.000	0.875	0.916	0.000
3	0.597	0.960	0.540	0.666	1.000	0.000
4	0.785	0.898	0.817	0.996	0.896	1.000
5	0.912	0.351	0.935	0.978	0.562	0.000
6	0.925	0.919	0.000	0.834	0.487	0.000
7	0.944	0.500	0.100	0.816	0.825	0.500
8	0.593	0.800	0.000	0.933	0.250	1.000
9	0.973	0.250	0.000	0.908	0.611	0.947
10	0.161	0.412	0.000	0.266	0.647	1.000
11	0.873	0.702	0.000	0.736	0.941	1.000
12	0.653	0.511	0.015	0.980	0.944	0.000
13	0.988	0.081	0.830	0.989	0.613	1.000
14	0.512	0.627	0.923	0.333	0.842	0.363
15	0.834	0.687	0.000	0.844	0.950	0.000
16	0.266	0.869	0.886	0.176	0.874	0.649
17	0.916	0.750	0.909	0.935	0.753	0.978
18	0.538	0.758	0.000	0.809	0.642	1.000
19	0.708	0.911	1.000	0.927	0.416	1.000
20	0.341	0.882	0.000	0.100	0.964	1.000
21	0.705	0.910	0.000	1.000	0.062	0.000
22	0.426	0.300	0.977	0.835	0.409	1.000
23	0.379	0.400	0.000	0.909	0.470	0.000

24	0.863	0.689	0.275	0.396	0.938	0.000
25	0.801	0.583	0.539	0.827	80.960	1.000
26	0.740	0.889	0.000	0.885	0.888	0.000
27	0.685	0.514	0.000	0.607	0.732	0.000
28	0.333	0.858	0.947	0.809	0.676	1.000
29	0.861	0.781	0.000	0.955	0.642	0.500
30	0.333	0.545	0.808	0.815	0.450	1.000
31	0.177	0.693	0.925	0.975	0.200	0.000
32	0.854	0.200	1.000	0.534	0.600	0.000
33	0.788	0.610	1.000	0.641	0.192	0.000
34	0.422	0.635	0.000	0.969	1.000	1.000
35	0.492	0.864	0.571	0.391	1.000	0.000
36	0.985	0.942	0.000	0.445	0.931	0.000
37	0.585	0.572	0.857	0.929	0.400	0.995
38	0.871	0.821	0.941	0.588	0.818	0.000
39	0.796	0.829	0.000	0.519	0.642	0.587
40	0.818	0.188	0.000	0.864	0.600	0.846
41	0.509	0.733	0.730	0.111	0.843	0.000
42	0.809	0.709	1.000	0.134	0.902	0.933
43	0.609	0.818	0.979	0.875	0.976	0.000
44	0.935	0.416	0.545	0.935	0.708	0.000
45	0.929	0.944	0.793	1.000	0.963	0.754
46	0.187	0.104	0.875	0.909	0.538	0.530
47	0.928	0.830	0.000	0.942	0.898	1.000
48	0.924	0.773	0.947	0.866	0.970	0.310
49	0.907	0.646	1.000	0.764	0.865	0.000
50	0.930	0.920	0.000	0.875	0.975	0.000
Average	**0.6983**	**0.65912**	**0.4637**	**0.74024**	**2.32692**	**0.45784**

27 February 2019						
Replicate	**Tank A**			**Tank B**		
Number	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**
1	0.645	0.496	0.723	0.840	0.968	0.400
2	0.923	0.800	0.000	0.129	0.881	1.000
3	0.880	0.727	0.943	0.954	0.864	0.000
4	0.876	0.263	1.000	0.357	0.937	0.000
5	0.850	0.537	1.000	0.764	0.949	0.000
6	0.707	0.708	0.000	0.656	0.809	0.000
7	0.756	0.524	1.000	0.562	0.750	0.000
8	1.000	0.889	0.000	0.931	0.179	0.880
9	0.294	0.505	0.000	0.757	0.992	0.000
10	1.000	0.815	0.640	0.967	0.534	0.977
11	0.934	0.917	0.579	0.595	0.936	1.000

12	1.000	0.373	0.962	0.784	0.929	0.000
13	0.279	0.859	0.666	0.550	0.696	1.000
14	0.302	0.699	1.000	0.861	0.918	1.000
15	0.719	0.872	1.000	0.687	0.910	0.500
16	0.684	0.369	0.000	0.113	0.611	0.000
17	0.702	0.820	0.000	0.536	0.638	0.000
18	0.340	0.899	1.000	0.757	0.744	0.000
19	0.444	0.871	0.000	0.457	0.934	0.000
20	0.711	0.545	0.963	0.690	0.799	0.987
21	0.629	0.949	0.519	0.742	0.954	0.750
22	0.965	0.521	0.966	0.944	0.824	0.000
23	0.879	0.787	0.000	0.453	0.260	0.000
24	0.631	0.131	0.846	0.683	0.793	0.000
25	0.731	0.865	0.675	0.765	0.762	0.000
26	0.285	0.961	0.732	0.700	0.936	0.000
27	0.990	0.711	0.825	0.925	0.962	0.923
28	0.627	0.667	0.000	0.439	0.926	1.000
29	0.027	0.984	0.000	0.626	0.500	0.000
30	0.535	0.647	0.123	0.942	1.000	0.971
31	0.830	0.878	0.621	0.864	0.890	1.000
32	0.642	0.987	0.000	0.960	0.985	0.000
33	0.271	0.933	0.958	0.924	0.916	0.000
34	0.786	0.927	0.066	0.783	0.126	0.000
35	0.892	0.793	0.000	0.895	0.943	0.000
36	0.605	0.937	0.098	0.828	0.974	0.000
37	0.937	0.828	0.097	0.820	0.994	0.416
38	0.211	0.218	0.865	0.894	1.000	0.798
39	0.446	0.800	0.939	1.000	0.951	0.833
40	0.347	0.955	0.000	0.989	0.928	1.000
41	0.487	0.435	0.964	0.500	0.154	0.000
42	0.943	0.829	0.000	0.350	0.853	0.866
43	0.900	0.688	0.896	0.958	0.934	0.384
44	0.506	0.854	0.300	0.542	0.819	0.705
45	0.956	0.931	0.015	0.250	0.771	0.000
46	0.888	0.246	0.378	0.964	0.922	1.000
47	0.584	0.773	0.622	0.521	0.380	0.000
48	0.895	0.694	0.633	0.925	0.787	0.000
49	0.762	0.722	0.000	0.638	0.863	0.000
50	0.367	0.877	0.979	0.968	0.723	0.000
Average	**0.672**	**0.72032**	**0.49186**	**0.71478**	**0.79616**	**0.3678**

6 March 2019						
Replicate	Tank A			Tank B		
Number	High	Medium	Low	High	Medium	Low
1	0.842	0.771	1.000	0.588	0.830	1.000
2	0.968	0.875	1.000	0.297	0.561	0.000
3	0.555	0.466	0.000	0.465	0.893	1.000
4	0.720	0.710	0.000	0.977	0.869	1.000
5	0.800	0.937	1.000	0.981	0.821	0.000
6	0.312	0.772	0.000	0.992	0.400	1.000
7	0.847	0.419	0.000	0.919	0.928	0.000
8	0.487	0.860	0.717	0.989	0.909	0.000
9	0.672	0.867	1.000	0.734	0.153	0.925
10	0.827	0.815	0.000	0.351	0.894	1.000
11	0.907	0.933	0.000	0.543	0.619	0.000
12	0.265	0.740	0.000	0.902	0.476	0.000
13	0.717	0.915	0.987	0.219	0.903	0.000
14	1.000	0.719	1.000	0.823	0.089	1.000
15	0.964	0.422	0.000	0.784	0.840	0.000
16	0.470	0.828	0.000	0.406	0.294	0.000
17	0.264	0.551	0.000	0.882	0.559	0.000
18	0.561	0.864	0.000	0.438	0.897	0.000
19	0.734	0.471	0.000	0.898	0.803	0.973
20	0.692	0.906	0.888	0.848	0.692	0.000
21	0.785	0.906	1.000	0.583	0.868	0.000
22	0.966	0.902	0.000	0.747	0.243	0.333
23	0.816	0.423	0.000	0.690	0.965	0.891
24	0.849	0.923	0.000	0.591	0.705	1.000
25	0.312	0.749	0.000	0.738	0.330	0.000
26	0.724	0.556	1.000	0.844	0.996	0.000
27	0.890	0.886	0.980	0.759	0.902	1.000
28	0.880	0.431	1.000	0.812	0.384	0.000
29	0.833	0.594	0.000	0.642	0.642	0.000
30	0.259	0.757	0.000	0.888	0.949	0.266
31	0.631	0.925	0.000	0.426	0.880	0.368
32	0.286	0.832	0.000	0.925	0.619	0.937
33	0.825	0.601	0.555	0.953	0.424	0.000
34	0.813	0.829	0.000	0.664	0.458	0.666
35	0.860	0.652	0.950	0.326	0.541	0.000
36	0.752	0.830	1.000	0.819	0.974	0.885
37	0.908	0.666	0.000	0.822	0.647	0.000
38	0.655	0.680	0.000	0.308	0.613	1.000
39	0.503	0.933	0.000	0.563	0.959	0.979
40	0.903	0.967	0.560	0.598	0.581	0.000
41	0.343	0.950	0.963	0.764	0.860	0.000

42	0.800	0.468	0.000	0.778	0.964	0.000
43	0.842	0.112	0.964	0.508	0.439	0.000
44	0.227	0.772	0.000	0.920	0.317	0.000
45	0.704	0.926	0.978	0.760	0.476	0.000
46	0.838	0.893	0.000	0.900	0.615	1.000
47	0.680	0.618	0.838	0.272	0.575	0.000
48	0.235	0.767	0.636	0.929	0.446	0.384
49	0.390	0.511	0.000	0.741	0.370	0.889
50	0.878	0.805	0.928	0.954	0.992	1.000
Average	**0.67982**	**0.7341**	**0.39888**	**0.7052**	**0.66328**	**0.38992**

13 March 2019						
Replicate	**Tank A**			**Tank B**		
Number	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**
1	0.760	0.658	0.000	0.478	0.833	0.000
2	0.968	0.397	0.000	0.904	0.500	0.000
3	0.943	0.408	0.000	0.703	0.812	0.000
4	0.848	0.945	0.000	0.802	0.984	0.000
5	0.466	0.848	0.000	0.947	0.635	0.000
6	0.929	0.671	0.000	0.655	0.698	0.000
7	0.924	0.355	0.000	1.000	0.352	0.000
8	0.840	0.870	0.000	1.000	0.755	0.000
9	0.752	0.992	0.000	0.904	0.990	0.000
10	0.517	1.000	0.000	0.808	0.947	0.000
11	0.922	0.988	0.000	0.813	0.947	0.000
12	0.512	0.500	0.000	0.828	0.348	0.000
13	0.607	0.298	0.000	0.429	0.930	0.000
14	0.881	0.946	0.000	0.840	0.693	0.000
15	0.780	0.761	0.000	0.800	0.761	0.000
16	0.460	0.259	1.000	0.376	0.783	0.000
17	0.874	0.811	0.833	0.953	0.842	0.000
18	1.000	0.644	0.000	0.408	0.796	1.000
19	0.633	0.942	1.000	0.733	0.683	0.000
20	0.949	0.858	0.000	0.745	0.763	0.750
21	0.847	0.994	0.000	0.984	0.765	0.985
22	0.645	0.803	0.000	0.517	0.175	0.000
23	0.781	0.666	0.000	0.506	0.703	0.000
24	0.875	0.703	0.000	0.946	0.768	0.972
25	0.380	1.000	1.000	0.530	0.658	1.000
26	0.818	1.000	0.000	0.828	1.000	0.000
27	0.880	0.963	0.000	0.619	0.452	0.000
28	0.568	1.000	1.000	0.929	0.571	1.000
29	0.277	0.905	1.000	0.742	0.601	1.000
30	0.866	0.316	0.000	0.885	0.100	0.000

31	0.896	0.825	0.000	0.869	0.500	0.000
32	0.978	0.903	0.666	0.969	0.886	0.000
33	0.339	0.970	1.000	0.809	0.968	0.000
34	0.821	0.858	0.000	0.952	0.871	0.000
35	0.794	0.757	1.000	0.925	0.166	0.000
36	0.855	0.278	0.000	0.419	0.962	0.000
37	0.347	0.974	0.000	0.729	0.181	0.000
38	0.827	0.593	0.000	0.444	0.945	1.000
39	0.482	0.775	1.000	0.807	0.973	0.000
40	0.945	0.853	1.000	0.552	0.586	0.913
41	0.725	0.655	0.000	0.704	0.985	0.000
42	0.624	0.856	0.000	0.911	0.911	1.000
43	0.638	0.892	0.000	0.921	0.506	0.000
44	0.531	0.828	1.000	0.795	0.852	1.000
45	0.850	0.855	1.000	0.236	0.994	0.000
46	0.826	0.867	0.442	0.978	0.257	0.000
47	0.518	0.466	0.987	0.803	0.317	0.000
48	0.886	0.283	0.981	0.984	0.759	1.000
49	0.633	0.696	1.000	0.536	0.702	0.000
50	0.903	0.401	0.000	0.421	0.594	0.000
Average	**0.7384**	**0.74172**	**0.31818**	**0.74752**	**0.6952**	**0.2324**

APPENDIX (C): STATISTICS

ANOVA tables

Seagrass growth ANOVA two way:

H1: There is no difference in mean YII between different weeks

H2: There is no difference in mean YII between the different light intensities.

H3: The light intensity does not have an impact on mean YII in different weeks

Tank A ANOVA:

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample (H1)	10.39918	8	1.299897	16.753	7.53E-24	1.945389	Reject
Columns (H2)	8.628473	2	4.314236	55.60163	0	3.002526	Reject
Interaction (H3)	5.972635	16	0.37329	4.810936	1.2E-09	1.651157	Reject
Within	102.6541	1323	0.077592				
Total	127.6544	1349					

Tank B ANOVA:

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample (H1)	10.25468	8	1.281834	16.12081	6.77E-23	1.945389	Reject
Columns (H2)	11.57313	2	5.786563	72.77391	0	3.002526	Reject
Interaction (H3)	10.82536	16	0.676585	8.508979	5.7E-20	1.651157	Reject
Within	105.1974	1323	0.079514				
Total	137.8505	1349					

High vs. medium tank B

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	1.599763	8	0.19997	4.127145	7.5E-05	1.948884	Reject
Columns	0.004301	1	0.004301	0.088762	0.765827	3.852024	Accept
Interaction	0.586102	8	0.073263	1.512054	0.148766	1.948884	Accept
Within	42.73507	882	0.048452				
Total	44.92524	899					

High vs low tank B

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	11.08033	8	1.385041	14.82425	1.75E-20	1.948884	Reject
Columns	8.870867	1	8.870867	94.94591	2.24E-21	3.852024	Reject
Interaction	8.668565	8	1.083571	11.59758	9.31E-16	1.948884	Reject
Within	82.40591	882	0.093431				
Total	111.0257	899					

Medium vs Low tank B

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	13.24194	8	1.655243	17.12446	8.19E-24	1.948884	Reject
Columns	8.48452	1	8.48452	87.77737	5.96E-20	3.852024	Reject
Interaction	6.983376	8	0.872922	9.030894	5.7E-12	1.948884	Reject
Within	85.25372	882	0.09666				
Total	113.9636	899					

High vs medium tank A

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	2.517464	8	0.314683	6.412136	4.03E-08	1.948884	Reject
Columns	0.002503	1	0.002503	0.051009	0.821369	3.852024	Accept
Interaction	0.285483	8	0.035685	0.727142	0.667641	1.948884	Accept
Within	43.28518	882	0.049076				
Total	46.09063	899					

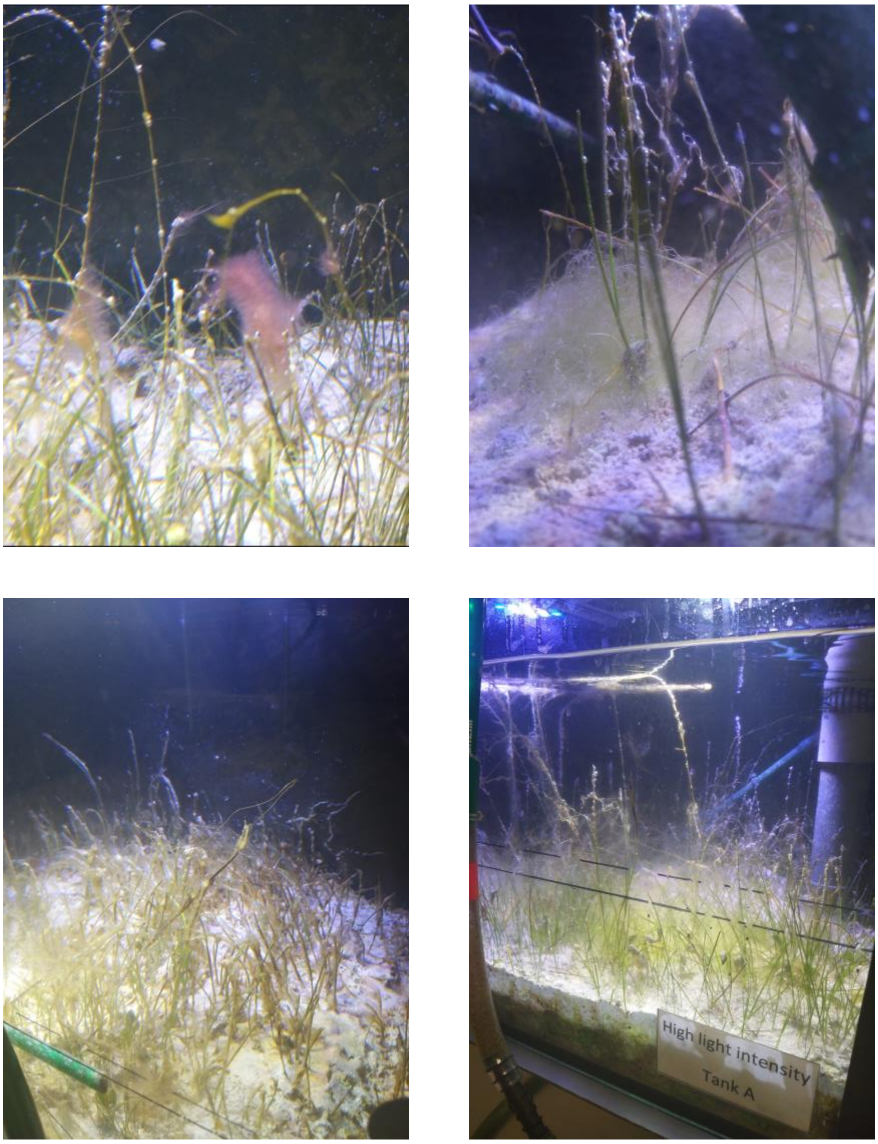
High vs low tank A

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	10.84978	8	1.356222	14.64403	3.2E-20	1.948884	Reject
Columns	6.342842	1	6.342842	68.48787	4.69E-16	3.852024	Reject
Interaction	4.022224	8	0.502778	5.428827	1.07E-06	1.948884	Reject
Within	81.68434	882	0.092613				
Total	102.8992	899					

Medium vs low tank A

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	10.41743	8	1.302179	14.29601	1.03E-19	1.948884	Reject
Columns	6.597363	1	6.597363	72.42934	7.38E-17	3.852024	Reject
Interaction	4.651246	8	0.581406	6.382979	4.45E-08	1.948884	Reject
Within	80.33864	882	0.091087				
Total	102.0047	899					

APPENDIX (D): PHOTOGRAPHS



Pictures showing the macroalgae growing on the seagrass in the medium (227 PAR) and high (452 PAR) light intensities.