EFFECTS OF LIGHT ON THE DISTRIBUTIONS, PHOTOSYNTHESIS AND RECOVERY OF *Halophila ovalis* (R. Br.) Hook. *f.* IN PULAU KOREA (PULAU GAZUMBO), PENANG

MICHELLE GLORY G JONIK

UNIVERSITI SAINS MALAYSIA

2020

EFFECTS OF LIGHT ON THE DISTRIBUTIONS, PHOTOSYNTHESIS AND RECOVERY OF *Halophila ovalis* (R. Br.) Hook. *f.* IN PULAU KOREA (PULAU GAZUMBO), PENANG

by

MICHELLE GLORY G JONIK

Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

April 2020

ACKNOWLEDGEMENT

It is a genuine pleasure to express my deep sense of thanks and gratitude to my main supervisors Dr. Sazlina Salleh and co-supervisor Dr. Mahadi Mohammad. Their dedication and keen interest above all their overwhelming attitude to help their students had been solely and mainly responsible for completing my thesis. Their timely advice, scholarly advice and scientific approach have helped me to a very good extent to accomplish my thesis writing. Special thanks to Dr. Zarul Hazrin Hashim for his guidance and ideas especially on statistics section of my thesis.

I also would like to extend my thankfulness to the most precious persons in my life, my beloved father and mother for all their moral support, words of encouragement, prayers and financial support and always there for me. I also owe a deep sense of gratitude to my dear friends and colleagues from Lab 209 especially Alia Nordin, Aysha Mariam, Aqilah Darif, Hana Marican, Nur Shakila Samad, Nur Ain Amani, Nadthikphorn A/P Kamphol, Mohammad Hilal Zainudin, Mohammad Firdaus Mutalib, Abdullah Amer, Sandy Mandy, Lucas Pipin, Bushy, Ahmad Johari for their keen interest on me at every stage of my research. Their prompt inspirations, timely suggestions with kindness, enthusiasm and dynamism have enabled me to complete my work.

Last but not least, million thanks to all that helped me doing my Master candidature and sampling trip. In addition, I would like to thank the staffs from Centre for Policy Research and International Studies (CenPRIS) and School of Biological Sciences for accommodating the workspace and facilities, providing conferences and workshops fundings. Not forgetting a big appreciation to the CenPRIS and Universiti

Sains Malaysia (USM) for giving me a golden opportunity to gain new knowledge in my Master research and awarding the *Prestigious External Award* and Graduate Assistant Scheme. I would like to acknowledge Malaysia Toray Science Foundation (MTSF) Science and Technology Research Grant (304.CDASAR. 650927.M126) and MOSTI Flagship Grant (304.CDASAR. 650724.P131) for funding my research.

Michelle Glory

TABLE OF CONTENTS

ACKNOWLEDGEMENT ..... **ii**

TABLE OF CONTENTS ..... **iv**

LIST OF TABLES ..... **viii**

LIST OF FIGURES ..... **xi**

LIST OF SYMBOLS AND ABBREVIATIONS ..... **xiv**

ABSTRAK ..... **xvi**

ABSTRACT ..... **xviii**

CHAPTER 1 GENERAL INTRODUCTION **1**

1.1 Overview of seagrass 1

1.2 Distribution and diversity of seagrasses in worldwide and Malaysia 4

1.3 Importance of seagrass in coastal ecosystems 5

1.4 Environmental factors affecting seagrass distributions and photosynthesis performance 7

1.4.1 Light availability 8

1.4.2 Water Temperature 8

1.4.3 Salinity 9

1.4.4 Sediment properties 10

1.4.5 Pore water nutrients 11

1.5 The application of Pulse Amplitude Modulated (PAM) in measuring seagrass photosynthesis 12

1.5.1 Chlorophyll fluorescence measurements 13

1.5.2 Fluorescence quenching pathway 17

1.5.3 Photosynthetic parameters determined from the Rapid Light Curves (RLCs) 18

1.6 Problem statement 20

1.7 Study objectives 22

1.8 Hypothesis 23

1.9 Thesis outline 24

CHAPTER 2 THE DISTRIBUTION AND PHOTOSYNTHESIS OF *Halophila ovalis* (R. Br) hook. *f* IN PULAU KOREA (PULAU GAZUMBO) ... 26

2.1 INTRODUCTION 26

2.1.1 Survival of seagrass under turbid water 26

2.1.2 Seagrass research and habitat in Penang coastal areas 28

2.2 MATERIALS AND METHODS 30

2.2.1 Study area 31

2.2.2 *In-situ* water parameter 35

2.2.3 Total suspended solid (TSS) 35

2.2.4 Pore water nutrients 36

2.2.4(a) Nitrite (NO_2^-) 37

2.2.4(b) Nitrate (NO_3^-) 37

2.2.4(c) Ammonia (NH_3) 39

2.2.4(d) Ortho-phosphate (PO_4^{2-}) 40

2.2.5 Sediment composition 40

2.2.6 Chlorophyll analysis 41

2.2.7 Identification of *Halophila* species in Pulau Korea 42

2.2.8 *Halophila ovalis* distribution in Pulau Korea 42

2.2.9 Seagrass biomass and morphometric growth analysis 44

2.2.10 Monitoring of *in*-situ chlorophyll fluorescence of *Halophila ovalis* 45

2.2.10(a) Effective and maximum quantum yield 47

2.2.10(b) Photosynthetic parameters derived from the rapid light curves (RLCs) 47

2.2.10(c) Non-photochemical quenching (NPQ) and photochemical quenching (qP) 49

2.2.11 Statistical analysis 49

2.3 RESULTS.....51

2.3.1 *In-situ* water physical parameters 51

2.3.2 Pore water nutrients 55

2.3.3 Sediment composition.....58

2.3.4 Diversity of seagrass in Pulau Korea 59

2.3.5 Distribution of *Halophila ovalis* in Pulau Korea 61

2.3.6 Morphometric growth and biomass of *Halophila ovalis* in Pulau Korea.....63

2.3.7 Total chlorophyll content of *Halophila ovalis*.....66

2.3.8 *In-situ* chlorophyll fluorescence measurements of *Halophila ovalis* using Pocket-PAM and Diving-PAM 67

2.3.8(a) Effective quantum yield (ΔF/Fm') and maximum quantum yield (F_v/F_m) 67

2.3.8(b) Rapid Light Curves photosynthetic parameters (RLCs)69

2.3.8(c) 2.3.8(c) Non-photochemical quenching (NPQ) and photochemical quenching (qP).....71

2.3.8(d) Canonical Correspondence Analysis (CCA).....74

2.3.9 The effects of *in-situ* environmental parameters on the distribution, morphometric growth, biomass and photosynthesis performances of *Halophila ovalis* in Pulau Korea.....76

2.4 DISCUSSION 81

CHAPTER 3 THE EFFECTS OF HIGH-LIGHT (HL) AND LOW-LIGHT (LL) ON THE PHOTOSYNTHESIS PERFORMANCE AND RECOVERY OF *Halophila ovalis* 90

3.1 INTRODUCTION.....90

3.1.1 Survival of seagrass under different light conditions.....90

3.2 MATERIALS AND METHODS 96

3.2.1 Plant materials collection.....96

3.2.2 Experimental set up.....98

3.2.3 Photo-physiology and dark-recovery analysis 100

3.2.1 Statistical analysis.....102

3.3 RESULTS 103

3.3.1 Effects of high-light (HL) and low-light (LL) on the photosynthesis and recovery of *Halophila ovalis* 103

3.4 DISCUSSIONS 108

CHAPTER 4 GENERAL DISCUSSION AND SUMMARY 112

CHAPTER 5 CONCLUSION 116

5.1 CONCLUSION 116

5.2 FUTURE RECOMMENDATION 118

REFERENCES 121

APPENDICES

LIST OF TABLES

		Page
Table 1.1	Basic characteristics of seagrasses according to their families summarized from Short & Coles (2001) and Kuo & Den Hartog (2001).	2
Table 1.2	The terminology used in fluorescence measurement (Adapted from Consalvey *et al*., 2005).	14
Table 2.1	Samples and data collection schedule throughout the monitoring period within year 2016 – 2017. Data taken in January 2016 are for the preliminary study. Data collections at site E was discontinued in 2017 due to very low seagrass distribution. The measurement of photosynthetic parameters were only initiated after PAM is available in February 2016 onwards.	34
Table 2.2	Mean rainfall (mm) in Penang from year 2016 to 2017 during sampling months.	34
Table 2.3	Braun-Blanquet Scale (Braun-Blanquet, 1972).	44
Table 2.4	Technical characteristics of Diving-PAM and Pocket-PAM (Adapted from Figueroa *et al*., 2013). Walz GmbH (Effletrich, Germany) and the Pocket-PAM by Gademann Instruments (Würzburg, Germany) produce diving-PAM. LEDs: light emitted diodes.	46
Table 2.5	Two-way Analysis of Variance (Two-way ANOVA) for significant effects of temporal changes (month) and spatial variations (site) on the water physical parameter. Bold indicates there is a significant effect ($P < 0.05$).	54
Table 2.6	Two-way Analysis of Variance (Two-way ANOVA) for significant effects of temporal changes (month) and spatial variations (site) on pore water nutrients. Bold indicates there is a significant effect ($P < 0.05$).	57
Table 2.7	Two-way Analysis of Variance (Two-way ANOVA) for significant effects of temporal changes (month) and spatial variations (site) on the sediment composition. Bold indicates there is a significant interaction ($P < 0.05$).	59

Table 2.8	Summary of morphological characteristics of *Halophila ovalis.*	60
Table 2.9	Two-way Analysis of Variance (Two-way ANOVA) for significant effects of temporal changes (month) and spatial variations (site) on the percentage coverage. Bold indicates there is a significant interaction ($P < 0.05$).	62
Table 2.10	Two-way Analysis of Variance (Two-way ANOVA) for significant effects of temporal changes (month) and spatial variations (site) on the morphology measurement and biomass data. Bold indicates there is a significant interaction ($P < 0.05$).	65
Table 2.11	Two-way Analysis of Variance (Two-way ANOVA) for significant effects of temporal changes (month) and spatial variations (site) on the total chlorophyll content. Bold indicates there is a significant interaction ($P < 0.05$).	66
Table 2.12	Two-way Analysis of Variance (Two-way ANOVA) for significant effects of temporal changes (month) and spatial variations (site) on the photosynthetic parameters. Bold indicates there is a significant interaction $P < 0.05$.	72
Table 2.13	Correlations, eigenvalues, and variance explained for the two axes of CCA for tested variables of *H. ovalis* (percentage coverage, morphometric sizes, biomass and all photosynthetic parameters) with environmental parameters and pore water nutrients based on monitoring months and sampling sites in Pulau Korea.	75
Table 3.1:	Samples and data collection schedule for High-Light (HL) and Low-Light (LL) experiments.	97
Table 3.2:	Range of *in*-situ environmental and photosynthetic parameters data recorded at the high-light (HL) and low-light (LL) collection sites within dry and wet seasons during sample collection. Photosynthetic parameters were obtained by using Diving-PAM (D) and Pocket-PAM (P). (-) indicates no data was taken in that sampling event.	97
Table 3.3	Environmental parameters recorded every three hours of under two light treatment; HL and LL. (Values are means ± S.E, n = 15)	99
Table 3.4	Two-way Analysis of Variance (Two-way ANOVA) for the significant effects of different light treatment (high-light and low-light) and exposure durations (*in*-situ, 1, 3 and 6 hours) on the photosynthetic parameters of *Halophila ovalis*. Bold indicates there is a significant interaction ($P < 0.05$).	106

Table 4.1 Summary of the *in*-situ and experiment environmental parameters and photosynthetic responses of *Halophila ovalis*. P indicates data was recorded by Pocket-PAM, while D was by Diving-PAM. (Values are ranges of data recorded). Note: (-) indicates there was no data recorded 115

LIST OF FIGURES

		Page
Figure 1.1	Guide to the identify some of the common tropical seagrass species (modifed from Di Carlo & McKenzie, 2011).	3
Figure 1.2	The theoretical diagram that explains the dark and light reaction of chlorophyll fluorescence output in Photosystem II from Consalvey *et al.* (2005).	16
Figure 1.3	Rapid light curve adapted from Ralph & Gademann (2006).	19
Figure 1.4	Thesis outline according to chapters.	25
Figure 2.1	Flow chart of summarized methodologies.	30
Figure 2.2	(a) Map of Peninsular Malaysia showing Pulau Pinang indicated by red square symbol. (b) and (c) are the location map showing the position of Pulau Korea in Straits of Penang indicated by red arrow and the sampling sites (A, B, C, D and E).	32
Figure 2.3	Layout of fixed transect point at every sampling site in Pulau Gazumbo.	33
Figure 2.4	Photo of quadrat used to estimate seagrass percentage cover. (Photo shows 50% seagrass coverage).	44
Figure 2.5	The mean value ±S.E. (n = 3) of *in*-situ water parameters (a) dissolved oxygen, (b) pore water pH, (c) salinity, (d) total suspended solid, (e) light intensity and (f) pore water temperature for every sampling site (A – E) throughout the monitoring months (January 2016 – November 2017) in Pulau Korea. Shaded area indicates dry months.	53
Figure 2.6	The mean value ±S.E. (n = 3) of pore water nutrients (a) nitrite, (b) nitrate, (c) ammonia, (d) ortho-phosphate, recorded in Pulau Korea for every sampling site (A – E) throughout the monitoring months (January 2016 – November 2017). Shaded area indicates dry months.	56
Figure 2.7	The mean value ±S.E. (n = 3) of sediment compositions in Pulau Korea for every sampling sites (A-E) throughout the monitoring months (January 2016 – November 2017). Shaded area indicates dry months.	58

Figure 2.8	Seagrass species found in Pulau Gazumbo throughout the monitoring period (a) *Halophila ovalis* (b) *Halophila beccarii* Asch and (c) *Enhalus acoroides* (L.f.) Royle.	61
Figure 2.9	The mean (± S.E) of seagrass percentage coverage (%) for every sites (A – E) throughout the monitoring months (January 2016 – December 2016) in Pulau Gazumbo. Shaded area indicates dry months.	62
Figure 2.10	The mean (± S.E) of *H. ovalis* morphometric measurement (a) above-ground morphometric measurement, (b) below-ground morphometric measurement and (c) the above and below-ground biomass of *H. ovalis* in Pulau Korea for every sampling sites (A-E) throughout the monitoring months (January 2016 – November 2017). Shaded area indicates dry months.	64
Figure 2.11	The mean value ± S.E of total chlorophyll (chlorophyll *a* + *b*) content per leaf area (μg Chl/cm²) of *Halophila ovalis* in Pulau Korea for every sampling sites (A - E) throughout the monitoring months (January 2016 – November 2017). Shaded area indicates dry months.	66
Figure 2.12	The mean value ± (S.E) of (a) maximum quantum yield (F_v/F_m) and (b) effective quantum yield ($\Delta F/F_m'$) of *Halophila ovalis* measured using Pocket-PAM (bar graph) and Diving-PAM (dotted line) at every sites (A – E) throughout the monitoring months (January 2016 – December 2016) in Pulau Korea. Shaded area indicates dry months.	68
Figure 2.13	The mean value ± (S.E) of RLCs photosynthetic parameters (a) maximum relative electron transport rate ($rETR_{max}$), (b) photosynthetic efficiency (α), (c) light saturation index (E_k) of *Halophila ovalis* measured using Pocket-PAM (bar graph) and Diving-PAM (dotted line) at every sites (A – E) throughout the monitoring months (January 2016 – December 2016) in Pulau Korea. Shaded area indicates dry months.	70
Figure 2.14	The mean value ± (S.E) of photosynthetic parameters (a) non-photochemical quenching (NPQ) and (b) photochemical quenching (qP) of *Halophila ovalis* measured using Pocket-PAM (bar graph) and Diving-PAM (dotted line) at every sites (A – E) throughout the monitoring months (January 2016 – December 2016) in Pulau Korea. Shaded area indicates dry months.	72

Figure 2.15	CCA ordinations incorporating relationships between all tested variables of *H. ovalis* with environmental parameters in Pulau Korea. Arrows correspond to scores of continuous variables (environmental parameters) and triangles are the tested dependent variables (percentage coverage, morphometric sizes, biomass and all photosynthetic parameters).	76
Figure 2.16	(a) Scatter plots of *Halophila ovalis* (a) percentage coverage with light intensity, (b) leaf length with nitrite, (c) leaf length with nitrate, (d) leaf length with ammonia, (e) maximum quantum yield (F_v/F_m) with light intensity, (f) effective quantum yield ($\Delta F/F_m'$) with light intensity, (g) $\Delta F/F_m'$ with water temperature, (h) maximum relative electron transport rate ($rETR_{max}$) with water temperature (i) photosynthetic efficiency (α) with light intensity, (j) light-saturating index (E_k) with light intensity, (k) E_k with seawater temperature, (l) E_k with chlorophyll content and (m) E_k with TSS as the significant affecting factors based on the linear stepwise multiple regression analysis.	78
Figure 3.1	Conceptual diagram of seagrass physiological responses under low-light and high-light conditions adapted from McMahon *et al*. (2013).	93
Figure 3.2:	High-light (HL) and low-light (LL) experimental and rate of recovery analysis set up.	101
Figure 3.3:	Variations of photosynthetic parameters (a) maximum quantum yield (F_v/F_m), (b) relative maximum electron transport rate ($rETR_{max}$), (c) photosynthetic efficiency (α), (d) light saturating index (E_k), (e) minimum fluorescence yield (F_o) and (f) non-photochemical quenching (NPQ) of *H. ovalis* treated under high light (HL) and low light (LL) conditions. Light treatment lasted for 6 hours continuously and the initial (0 hour) was measured before exposing the sample to the light treatment. *H. ovalis* leaves were randomly collected after every 1, 3 and 6 hours of exposure and subjected to five different dark-recovery time (0, 10, 15, 30 and 60 minutes). (Values are means ± S.E, n = 15).	107

LIST OF SYMBOLS AND ABBREVIATIONS

°C	degree Celsius
ppt	part per thousands
F_o	minimum fluorescence yield
F_m	maximum fluorescence yield during the saturating flash
F_v	variable fluorescence
Ft	fluorescence yield in the light adapted state
$F_{m'}$	fluorescence yield in the light adapted state during the saturating flash
F_v'	fluorescence quenched
F_v/F_m	maximum quantum yield of PSII
$F_v'/F_{m'}$	effective quantum yield
rETR	relative electron transport rate
rETRmax	maximum relative transport rate
α	photosynthetic efficiency
β	photoinhibition
E_k	photoacclimation index
qP	photochemical quenching
NPQ	non-photochemical quenching
D.O	dissolved oxygen
m	metre
mg	milligram
mg/L	milligram per litre
mL	millilitre
L	litre
μmol m^{-2} s^{-1}	micro mol metre per second

NO_3^-	nitrate
NO_2^-	nitrite
NH3	ammonium
PO_4^{2-}	phosphate
r	Pearson’s correlation coefficient
ANOVA	Analysis of variance
PAM	Pulse-Amplitude Modulation
PAR	Photosynthetically Active Radiation
PSI	Photosystem I
PSII	Photosystem II
RLC	Rapid Light Curve
SPSS	Statistical Package for Social Science
TSS	Total Suspended Solids

KESAN CAHAYA TERHADAP TABURAN, FOTOSINTESIS DAN PEMULIHAN *Halophila ovalis* (R.Br.) Hook. *f.* DI PULAU KOREA (PULAU GAZUMBO), PENANG

ABSTRAK

Daripada 72 spesies kepelbagaian global, terdapat 16 spesies dari genera yang berbeza tercatat di seluruh 78 tempat di Malaysia. Sehingga kini terdapat enam habitat rumput laut dilaporkan di wilayah utara Semenanjung Malaysia dan salah satunya adalah tapak kajian ini, Pulau Korea, Pulau Pinang. Tapak kajian ini kaya dengan kepelbagaian biologi dengan ramai masyarakat pesisir yang bergantung kepadanya sebagai sumber kehidupan. Walau bagaimanapun, Pulau Pinang adalah bandaraya yang kian membangun di Utara Semenanjung Malaysia dan Perbadanan Pembangunan Negeri telah mencadangkan untuk menambak Pulau Korea. Namun, dengan semua perkembangan pesisir bersebelahan dengan tapak kajian yang akan mengganggu kualiti air, kajian mengenai keadaan rumput laut dari segi fisiologi foto atau pengedaran adalah terhad. Oleh itu, kajian ini adalah untuk menyediakan laporan terkini dalam pengedaran, biojisim dan foto-fisiologi *Halophila ovalis* di persisiran Pulau Korea. Pengagihan rumput laut dianggarkan berdasarkan peratusan litupan, manakala tahap kesihatan dipantau melalui prestasi fisiologi foto dengan menggunakan teknik pendarfluor klorofil. Hasil kajian menunjukkan jumlah kawasan rumpai laut di Pulau Korea adalah kira-kira 4.49 ekar (39.8 ± 2.03%) dan diklasifikasikan sebagai liputan sederhana dengan skor skala Braun-Blanquet 3. Walau bagaimanapun, populasi rumput laut di Pulau Korea adalah rendah, dengan hanya 3 spesies rumput laut yang dikenal pasti (*H. ovalis* sebagai rumput laut dominan, *H. beccarii* dan *Enhalus acoroides*) dibandingkan dengan Kawasan rumput laut di

Merambong, Johor yang mempunyai lebih banyak spesies rumput laut. Perubahan edaran *H. ovalis* selama 11 bulan di Pulau Korea dipengaruhi secara negatif oleh perubahan temporal dalam keamatan cahaya (R^2 = -0.135). Tiada pengaruh signifikan daripada semua parameter alam sekitar terhadap pertumbuhan biojisim dan ciri morfologi *H. ovalis* kecuali panjang daun yang dipengaruhi oleh nitrit (R^2 = 0.114), nitrat (R^2 = 0.135) dan ammonia (R^2 = 0.044). Variasi dalam F_v/F_m *H. ovalis* dipengaruhi oleh intensiti cahaya (R^2 = -0.171). ΔF/Fm' dipengaruhi oleh intensiti cahaya (R^2 = 0.234) dan suhu (R^2 = 0.061). $rETR_{max}$ dan α. *ovalis* dipengaruhi secara positif oleh suhu air (R^2 = 0.112) dan intensiti cahaya (R^2 = 0.168) masing-masing. E_k dipengaruhi secara signifikan dari intensiti cahaya (R^2 = 0.091), suhu air (R^2 = 0.053), pepejal dalam tiang air (R^2 = -0.051) dan kandungan klorofil (R^2 = -0.066). Rumput laut sering terdedah kepada rejim cahaya yang berlainan akibat perubahan air pasang surut. Semasa pasang surut, rumpai laut di persisran terdedah kepada tekanan haba, pengeringan dan kerosakan-foto akibat pendedahan yang berpanjangan. Untuk memahami mekanisme kehidupan rumpai laut di Pulau Korea dan keupayaan daya tahan keseluruhannya untuk pulih daripada tekanan cahaya, analisis pemulihan gelap dilakukan di bawah pendedahan cahaya tinggi (HL) dan cahaya rendah (LL). Hasil kajian menunjukkan bahawa, sepanjang 6 jam pendedahan kepada rawatan HL (1052.7 - 2572.7 μmol foton m^{-2} s^{-1}), *H. ovalis* menunjukkan nilai NPQ yang tinggi (2.592 - 3.464) dan memerlukan tempoh masa 15 hingga 30 minit untuk mencapai nilai Fo dan Fv / Fm (0.735 - 0.854) yang tinggi dengan penurunan NPQ (1.286 - 3.065) sebanyak 30%. Di bawah rawatan LL (61.9 - 84.6 μmol foton^{-2} s^{-1}), tanpa mengambil kira masa pendedahan, penyesuaian gelap selama 10 minit telah cukup untuk pemulihan pusat reaksi *H. ovalis* dengan mempunyai nilai Fv/Fm sekitar 0.347 - 0.437, lebih tinggi daripada kawalan (0 minit pemulihan gelap; Fv/Fm: 0.263 - 0.309).

EFFECTS OF LIGHT ON THE DISTRIBUTIONS, PHOTOSYNTHESIS AND RECOVERY OF *Halophila ovalis* (R. Br.) Hook. *f*. IN PULAU KOREA (PULAU GAZUMBO), PENANG

ABSTRACT

From the 72 global species diversity of seagrass, there are 16 species from different genera were recorded throughout the 78 sites in Malaysia. To date, there are six seagrass habitats reported in the northern region of Peninsular Malaysia and one of it is this study site, Pulau Korea, Penang. The study site is rich in biodiversity with a significant number of coastal communities relying on it for their livelihood. However, Penang Island is a highly urbanised city state in Northern region of Malaysia and Penang States Development Corporation had proposed to reclaim Pulau Korea. Despite all the coastal developments adjacent to the study site which will disrupt the water quality, there have been limited studies of seagrass conditions in terms of photo-physiology nor the recent distribution are since year 2016. Thus, this study was to provide an updated report on the distribution, biomass and photo-physiology of *Halophila ovalis* in the intertidal zone of Pulau Korea. The distribution was estimated based on seagrass percentage cover, while the health of seagrass was monitored through the photo-physiological performance by using chlorophyll fluorescence technique. The findings indicated that the total estimated area of seagrass area in Pulau Korea was approximately 4.49 acres (39.8 ± 2.03%) and was classified as moderate coverage with Braun-Blanquet scale score 3. However, seagrass population in Pulau Korea was low, with only 3 identified seagrass species (*H. ovalis* as the dominant seagrass, *H. beccarii* and *Enhalus acoroides)* as compared with other seagrass bed in Merambong, Johor that have more seagrass species. The distribution variation of *H.*

ovalis over 11 months in Pulau Korea was negatively influenced by temporal changes in light intensity (R^2 = -0.135). No significant influence ($P > 0.05$) from all the environmental parameters was observed in biomass and morphology growth characteristics of *H. ovalis* except for leaf length that were influenced by nitrite (R^2 = 0.114), nitrate (R^2 = 0.135) and ammonia (R^2 = 0.044). Variations in F_v/F_m of *H. ovalis* was influenced by the light intensity (R^2 = -0.171). While, $\Delta F/F_m'$ was influenced by light intensity (R^2 = 0.234) and temperature (R^2 = 0.061). $rETR_{max}$ and α of *H. ovalis* were positively influenced by water temperature (R^2 = 0.112) and light intensity (R^2 = 0.168) respectively. Meanwhile significant influence from the light intensity (R^2 = 0.091), water temperature (R^2 = 0.053), TSS in water column (R^2 = -0.051) and chlorophyll content (R^2 = -0.066) have resulted in variations of E_k. Moreover, seagrasses are often exposed to different light regimes due to tidal changes. During low tide, intertidal seagrasses are highly vulnerable to thermal stress, desiccation and photodamage due to prolonged exposure. Therefore, to understand the survival mechanism of seagrass in Pulau Korea and its overall resilience ability to recover from light stress, a dark-recovery analysis was conducted under high-light (HL) and low-light (LL) exposure. Findings show that, throughout the 6 hours exposure to HL treatment (1052.7 – 2572.7 μmol photon m^{-2} s^{-1}) *H. ovalis* showed high NPQ values (2.592 – 3.464) which required 15 to 30 minutes dark-recovery period to achieve high F_o values and high F_v/F_m (0.735 – 0.854) with reduced NPQ (1.286 – 3.065) by 30%. Under LL treatment (61.9 – 84.6 μmol photon m^{-2} s^{-1}), regardless of the prolonged exposure, 10 minutes dark-adaptation is sufficient for the recovery of *H. ovalis* reaction centre by having F_v/F_m values around 0.347 – 0.437 which are higher than the control (0 minute dark-recovery; F_v/F_m: 0.263 – 0.309).

CHAPTER 1

GENERAL INTRODUCTION

1.1 Overview of seagrass

Seagrasses are aquatic flowering vegetations that able to complete their lifecycle while fully submerged in seawater (Björk *et al*., 2008). They are placed under the division of Magnoliophyta (Angiosperm) with six common families of seagrass found throughout the oceans that include Cymodoceaceae, Hydrocharitaceae, Posidoniaceae and Zosteraceae. Apart from the families mentioned before which have altogether 12 marine genera (Table 1.1), there are no other genera that are fully confined to the marine environment. In other aquatic plant, there are two families have been found that occur in eurysaline habitat which are Ruppiaceae, and Zannichelliaceae. Altogether there are 17 genera of seagrass under these 6 families (Short & Coles, 2001; den Hartog, 1970 cited from Kuo & den Hartog, 2001) and the basic characteristics to identify seagrass according to their families are as shown in Table 1.1 (Short & Coles, 2001; den Hartog, 1970 cited from Kuo & den Hartog, 2007). In traditional method, the characteristics used to identify and classifying flowering plants are the reproductive structures, for examples the petals, sepals, stamens, fruits and seeds. However, flowers and fruits of most seagrasses are not often collected. Therefore identification of seagrass species is more dependent on vegetative characteristics (Figure 1.1), such as leaf width, leaf tips, numbers of leaf vein, fibre distributions, epidermal cells, characteristics of the roots and rhizomes (Di Carlo & McKenzie, 2011).

Table 1.1: Basic characteristics to identify seagrass with respect to its families summarized from Short & Coles (2001), Kuo & den Hartog (2001) and Short *et al*. (2007).

Family	Genera	Characteristics
Marine environment		
Cymodoceaceae	• *Halodule* • *Cymodocea* • *Syringodium* • *Thalassodendron* • *Amphibolis*	Dioecious marine plants. Unisexual plants, without a perianth, solitary or in pairs, or arranged within a cymose inflorescence. Tannin cells present and generally male flower is sessile.
Hydrocharitaceae	• *Thalassia* • *Halophila* • *Enhalus*	Dioeciously or monoecious plants, with a trimerous perianth. Have round shape pollen, free or arranged within a moniliform string.
Posidoniaceae	• *Posidonia*	Having cymose type inflorescence, with the branches ending in 'spikes'. bisexual plants and normally containing three stamens with large connectives and one ovary.
Zosteraceae	• *Zostera* • *Heterozostera* • *Phyllospadix*	Dioecious or monoecious. Creeping, monpodial branching rhizomes with unbranched roots and one shoot at each node. Flowers without periant, arrayed in two rows on one side of a flattened spadix which enclosed in a spathe are either unisexual or bisexual. The ovary parallel with a short, thick style and two stigmata shed after flowering.
Eurysaline environment (water with unstable salinity)		
Ruppiaceae	• *Ruppia*	Monoecious plants, in pairs on a peduncle, each with two anthers and many ovaries, without a perianth.
Zannichelliaceae	• *Zannichellia* • *Lepilaena* • *Althenia* • *Pseudalthenia*	Very narrow leaves, with one central nerve, without tannin cells and consist of terminal flowers. Male flower consisting of a stalked anther and with small scaly perianth while the female flower could consist up to 8 free carpels on a joint pedicel, surrounded by a scaly perianth.

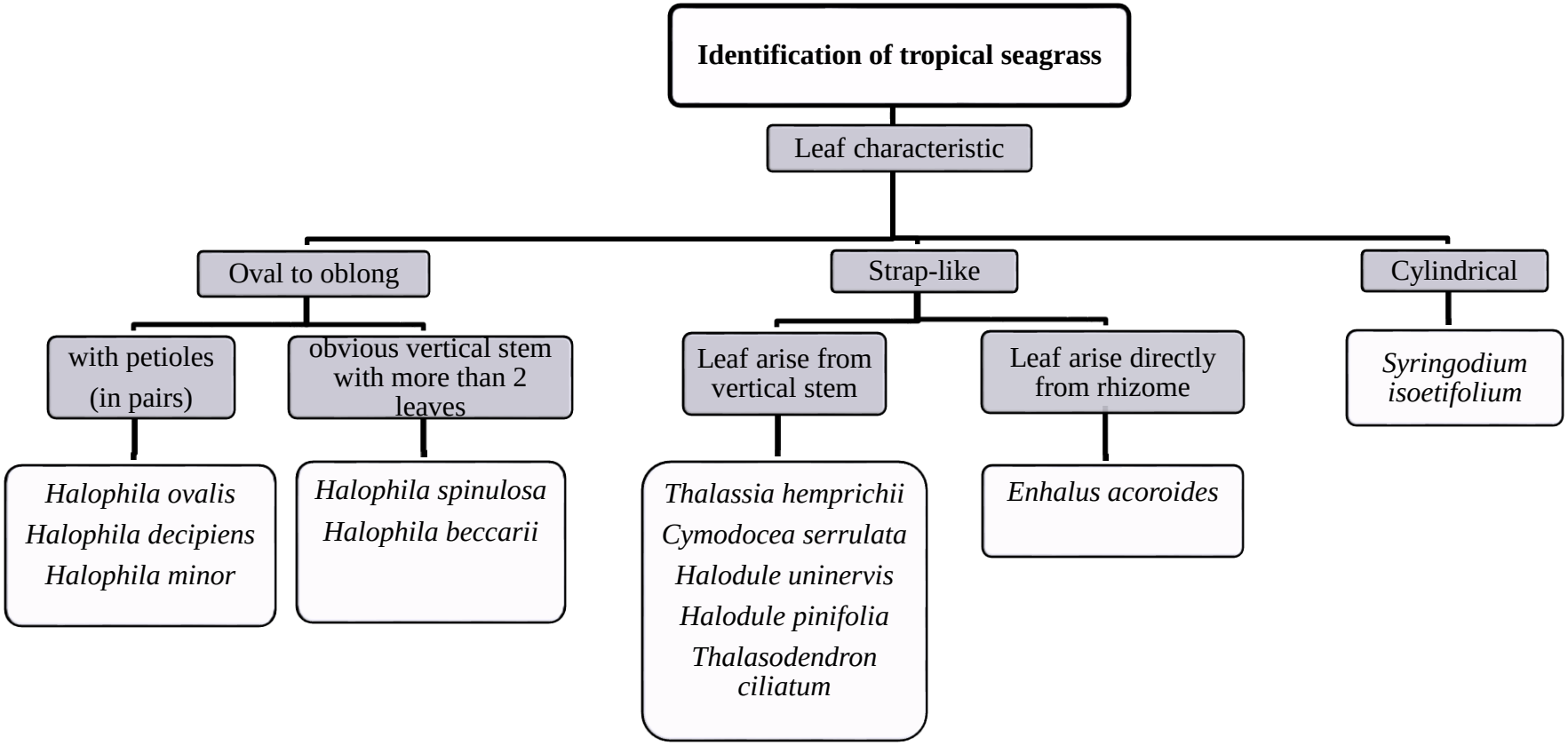

Figure 1.1: Guide to the identify some of the common tropical seagrass species (modifed from Di Carlo & McKenzie, 2011).

1.2 Distribution and diversity of seagrasses in worldwide and Malaysia

Seagrass can be further categorized according to their origin, either from temperate or tropical bioregions. Tropical seagrasses include Hydrocharitaceae (*Halophila, Thalassia* and *Enhalus*) and Cymodoceaceae (*Syringodium, Halodule,* and *Cymodocea*). While seagrasses in the temperate region are mainly from Cymodoceaceae (*Amphibolis*), Zosteraceae (*Zostera,* and *Phyllospadix*) and Posidoniaceae (*Posidonia*). *Thalassodendron* and *Ruppia* can be found in both temperate and tropical regions (Kuo & den Hartog, 2001; Short *et al*., 2007; Bujang *et al*., 2016). They are widely distributed throughout the world and form extensive meadow in every coastal zone continent except Antarctica with estimated coverage approximately 177,000 km^2 (Waycott *et al*., 2009). They could exist either in patches or extensive dense seagrass meadow that comprised of a monospecies or mixed species seagrass bed (Hemminga & Duarte, 2000; Kuo & Den Hartog, 2001). Worldwide, there are 72 seagrass species from 17 genera reported across both tropical and temperate waters (Short *et al*., 2007; Short *et al*., 2011) with the highest number of seagrass species (24 species) found in the Indo-Pacific Tropical region (Short *et al*., 2011; Coles *et al*., 2011). The most diverse seagrass species in the Indo-Pacific Tropical region is in Southeast Asia with 21 species of seagrasses and approximately 36,762.6 km^2 of seagrass area (Fortes *et al*., 2018). From the 21 seagrass species found in Southeast Asia, 11 of them are from the genus *Halophila* (Short *et al*., 2010; Fortes *et al*., 2018).

While in Malaysia, 78 sites of seagrass areas were recorded in Peninsular Malaysia, Sabah and Sarawak (Bujang *et al*., 2006). Most of the seagrasses in Malaysia

were found growing near shallow inter-tidal, mangroves, coral reefs, semi-closed lagoons, and shoals' areas (Bujang *et al*., 2006). Overall, there are 16 species of seagrasses (Bujang *et al*., 2016; Nguyen *et al*., 2014) were found along the 4800 km coastline extent that makes up a total of 16.3 km^2 seagrass area (Bujang *et al*., 2016; Abdullah & Anscelly, 2015; Fortes *et al*., 2018). Seagrasses that were found in Malaysia are *Enhalus acoroides*, *Halophila beccarii*, *Halophila decipiens*, *Halophila ovalis*, *Halophila major*, *Halophila minor*, *Halophila spinulosa*, *Halophila major*, *Halodule pinifolia*, *Halodule uninervis*, *Cymodocea rotundata*, *Cymodocea serrulata*, *Thalassia hemprichii*, *Syringodium isoetifolium*, *Thalassodendron ciliatum*, and *Ruppia maritima* (Bujang *et al*., 2016).

1.3 Importance of seagrass in coastal ecosystems

Seagrasses are considered to be one of the essential components of the oceans, but the seagrass meadows are declining globally due to anthropogenic threats (Short *et al*., 2011). Regardless of latitude differences, seagrasses are often considered a critical compound of marine and coastal environments as it is one of the most valuable biological system. They fulfil several ecological services within the marine ecosystem such as nursery, breeding and feeding grounds to numerous fish and invertebrate species (Arshad *et al*., 2005; Ara *et al*., 2011; Kimirei *et al*., 2011). The physical structures of seagrass leaves reduce the hydrodynamic activity by reducing water-currents and wave actions (Folmer *et al*., 2012) so that the early life stages of fish can settle in the water as well as provides protection to juvenile fish from predators. In addition, the abundance of food source in seagrass bed such as epiphytes, macro- and meio-fauna, as well as organic matter

through nutrient cycles in coastal ecosystem attract large numbers of other organisms. Moreover, the local coastal communities rely on seagrass meadows for source of food and livelihoods especially in tropical region. Invertebrate gleaning on the seagrass bed is a popular fishing method by the coastal communities to collect invertebrates at the intertidal areas during low tide and sell it to the fish market. (de la Torre-Castro & Rönnbäck, 2004; Nordlund *et al*., 2010, Cullen-Unsworth *et al*., 2014). Besides, the local entrepreneurs in tropical region also use seagrass to make high quality furniture and woven handicrafts for sale (Abad, 2008). In addition, seagrasses also play an important functional role in stabilizing sediments that provides protection from coastal erosion during storms as the dense roots and rhizomes form a secure mat that stabilizes the sea floor (Folmer *et al*., 2012; Kombiadou *et al*., 2014). Thus, the sediment resuspension can be reduced and this increase the water clarity. Hence, Penang's intertidal seagrass beds are generally crucial in maintaining coastline stability and reducing turbidity.

Seagrass is also known as an effective natural ecosystem carbon sequestration mainly the below-ground biomass (Alongi *et al*., 2016; Rozaimi *et al*., 2017). As the biomass decompose slowly, suspended organic carbon in the tissue will be buried for time scales and thus make seagrass meadows as efficient long-term carbon storage (Fourqurean *et al*., 2012; Lavery *et al*., 2013; Ganguly *et al*., 2018). Moreover, since seagrasses are sensitive to pollution or its side-effects, they are good marine biological indicator and are frequently used as early detection of increasing anthropogenic influences in coastal ecosystems (Orth *et al*., 2006; Ahmad *et al*., 2015). This can be measured from the changes in the distribution of some seagrass species as response to the environmental changes such as changes in water quality (Van Katwijk *et al*., 2011) or temperature

(Collier & Waycott, 2014), accumulation of heavy metal (Nguyen *et al*., 2017a; Nguyen *et al*., 2017b) and eutrophication in the coastal water (Gladstone-Gallagher *et al*. 2018; Yang *et al*., 2018).

1.4 Environmental factors affecting seagrass distributions and photosynthesis performance

The habitat of most seagrass meadows from intertidal-to-shallow-subtidal zone allows easy access to the local seagrass communities. As a result, it exposes the seagrass ecosystems to anthropogenic and natural disturbances (Short *et al*., 2011; Nordlund & Gullström, 2013; Cullen-Unsworth and Unsworth, 2016). Over the last 20 years, the losses of seagrasses have been substantial. Past reports indicated that the global losses of seagrass is alarming and at a declining rate of <1% per year since in the early year of 1980 which translated to a loss of-seagrass bed-at the rate of 110 km² per year or at a median rate of 5% – 7% per year (Borum *et al*., 2004; Waycott *et al*. 2007; Waycott *et al*. 2009). Globally, the estimated losses caused by human impact corresponds to 33 000 km^2 which is equivalent to 18 % of the total populated seagrass habitat documented. Therefore, in recent years, factors that controlling seagrass photosynthesis are getting more interest from the scientific community due to their ecological and economic value. The growth and species distribution of seagrass are manipulated by biotic and abiotic variables. These variables have impacts on the metabolism and the physiological process of seagrass (Lee *et al.*, 2007). Seagrass distribution, photosynthesis process, physiology and morphology are mainly controlled by abiotic factors such as temperature, light, and nutrient availability, as well as biotic factors such as competition between species and herbivory. Seagrass

distribution cannot be determined by a single factor, but rather multiple factors that are often tightly related. In addition, seagrass growth also require a suitable substratum and a generally low degree of physical exposure to waves, currents and tides.

1.4.1 Light availability

Seagrasses are very sensitive to reduction in light availability and considered to be species with highest light requirements amongst the flowering plants (Ralph *et al*. 2007). The depth limits for seagrass existence is likely to be controlled by light availability for photosynthesis. Generally most seagrasses are found in shallow water of less than 10 m as they required approximately 2 - 37% of surface irradiance to survive for a long period of time (Ralph *et al*., 2007). The availability of light is often associate with other factors such as (a) water depth (Ralph *et al*. 2007), (b) epiphytes shading (Drake *et al*., 2003; O'neil *et al*., 2012) and (c) suspended sediments (Zakaria & Bujang, 2011 cited in Hossain *et al*., 2015, Abdullah & Anscelly, 2015). However, some species can be found in exceptionally only clear water as deep as 61m to 70m in coastal tropical Indo-Pacific especially *Halophila* species particularly *H. ovalis*, *H. spinulosa, H. decipiens,* and *H.* tricostata (Short *et al.*, 2007; Coles *et al*., 2009). Most of the large seagrass species such as *Enhalus acoroides* has high tolerance to light deprivation compared to small seagrass species *H. ovalis* (Yaakub *et al*., 2014a).

1.4.2 Water Temperature

Water temperature along with light, serve as an important factor in controlling the productivity of marine organism (Collier *et al*., 2011; Said, 2017). Shallow growing

seagrasses in tropical region are often exposed to desiccation and temperature fluctuation especially at low tide (Rasheed & Unsworth, 2011). Although light absorption is independent of temperature, many of the photosynthesis reaction associated with the enzymes of Calvin cycle are temperature dependent. According to Lee *et al*. (2007), the ideal temperature for temperate species range from 11.5°C to 26°C, while for tropical and subtropical seagrass ranges from 23°C to 32°C. However, some tropical seagrass such as *H. ovalis* are able to tolerate water temperatures up to 40°C for a shorter period (2 days), while growth reductions and shoot were observed when exposed further (Collier & Waycott, 2014). Prolonged exposure to elevated temperatures (43°C), however, results in mortality in some species such as *Cymodocea rotundata*, *Halodule uninervis*, *H. ovalis* and *Thalassia hemprichii* (Collier & Waycott, 2014). Elevated temperature at 40°C to 43°C will reduce the photosynthetic performance and cause irreversible damage to the Photosystem II (PSII) (Collier & Waycott, 2014). When temperature rised above the threshold limit, carbon production in plants will be inhibited as respiration exceeds photosynthesis which resulting in a negative energy balance (Campbell *et al*., 2006).

1.4.3 Salinity

Most aquatic photosynthetic organism are also affected by the changes in salinity (Rudnick *et al*., 2005). Fluctuations in salinity will alter the photosynthetic pigments in some seagrasses and cause a decline in growth and reduce the photosynthetic rate (Sandoval-Gil *et al*., 2012). In the subtropics and tropics, the long-term sustainability of seagrass depends on their ability to adapt in salinity regimes (Koch *et al*., 2007). Even though salinity influences the osmotic pressure in the cells, most seagrass are well adapted

to fluctuation of salinity and some species may have a higher tolerance to salinity changes than others. According to Touchette (2007), most seagrass species preferred salinity ranges from 15 to 42 practical salinity units (PSU), except for *Ruppia* species that is usually found in fresh water (0 PSU). However, *H. ovalis* was seen to be able to tolerate a wide range of osmotic fluctuations and rapid changes in salinities from 9 PSU to 52 PSU without significant changes in photosynthetic response (Touchette, 2007). The optimum salinity for *H. ovalis* is 36 PSU and mortality may occur when salinity is below 6 PSU (Collier *et al*. 2014). Under hypo-salinity condition which is below 30 PSU, a stress-induced morphometric response will occur in *H. ovalis* with increased shoot density and leaf area as well as reduced sexual reproduction. However, seagrass studies in Malaysia particularly on *H. ovalis* were often found to grow well in salinities between 29 to 31 ppt (Annaletchumy *et al*. 2005).

1.4.4 Sediment properties

The nature of the sedimentary substrata in which seagrass root systems and rhizomes grow varies from coarse sand to mud, but some species can be found on more rocky substrates; such as *Phyllospadix* family (Spalding *et al*., 2003; Short *et al.*, 2016). It is important for seagrass to grow in suitable substrate as sediment provides not only a medium in which to anchor, but also a substratum in which complex chemical and microbial processes occur related to nutrient and gaseous recycling (Short *et al*., 2016). Sediment that generally contains mud, sandy and gravel may affect the growth responses of seagrass and nutrient enrichment (Short *et al*., 2016). Seagrass also could make some changes on the sediment composition by making it either sandier or muddier depending

on the density of seagrass coverage (van Katwijk *et al*., 2010). Sediment compositions that has high percentage of sand are likely to lose nutrients from pore water compared to the one that has high percentage of mud (Corbett, 2010). By comparison, sediment with higher percentage of mud tend to retain nutrients but more toxic due to lower interaction with the overhead water column (Koch, 2001) and generally have higher levels of porewater ammonia. Moreover, the characteristics of the sediment also define the surrounding environmental parameters of seagrass meadows (van Katwijk & Wijgergangs, 2004). Higher diffusion of oxygen will occur in sandier sediments that will inhibit fertility by constricting rhizome growth (McKenzie, 2007). Moreover, in the reefs habitat which are loaded with carbonate sediments, phosphorus tends to bind to the carbonate particles and limit the growth of seagrass; therefore, the size of seagrass in reef habitat is smaller compared to the other habitat (Fourqurean *et al*., 1992). Sediment grain size is often correlated with the water currents and tidal wave. Higher proportion of coarser sediments (sand and gravel) are found in high-energy water environments that experienced strong waves and fast-moving currents; while finer sediments are usually found in low-energy environments. Hence, seagrasses are mostly found in low-energy environments (Madsen *et al*. 2001).

1.4.5 Pore water nutrients

Seagrasses are able to take up nutrients from the ambient water column and pore water through either above-ground or below-ground tissues (Lee *at al*. 2007; Borum *et al*., 2004). The main nutrients such as nitrogen (N) and phosphorus (P) will stimulate growth in seagrass when present in moderate level (Connolly, 2009). Fluctuations in

nutrient levels occur naturally mainly due to seasonal weather changes (Hughes 2016). Generally, nutrient levels in the water column of seagrass beds are typically low, especially in warmer areas such as in Mediterranean and tropical regions (Greve & Binzer, 2004; Ahmad-Kamil, 2013). However, seagrasses usually take up nutrients from the sediment pore water as the nutrient concentrations are much higher than the overlying water column (Lee *et al*., 2007). Usually, seagrasses will be dominant over macroalgae in water column that has low nutrient concentrations due to the competitive advantage conferred by roots and rhizomes in absorbing nutrients from sediment porewater (Lee *et al*., 2007; Vonk *et al*., 2008). In contrast, the competitive interaction is reversed under high nutrient concentrations in water column since macroalgae are able to absorb nutrients more effectively through the thallus than seagrasses through their leaves (Lee *et al*., 2007; Vonk *et al*., 2008). However, high level of nutrients in the water column will stimulate the growth of phytoplankton and epiphytic algal growth on seagrass leaf surfaces, which reduced the light, inorganic carbon and availability for growth (O'neil *et al*. 2012; Bakker *et al*. 2010; Hauxwell *et al*. 2003).

1.5 The application of Pulse Amplitude Modulated (PAM) in measuring seagrass photosynthesis

Before Pulse Amplitude Modulated (PAM) was widely used, photosynthesis of seagrass was measured via the gas-exchange method (Hena *et al*., 2001a; Hena *et al*., 2001b). The *in-situ* measurements of photosynthetic activity in seagrass became possible after the development of PAM Fluorometry by using chlorophyll fluorescence technique. The chlorophyll fluorescence technique has been widely used in photosynthesis related

studies. PAM has been developed to assess the photosynthetic physiology in higher plants and later been applied to numerous aquatic autotrophs (Juneau *et al*., 2005; Salleh & McMinn, 2011; Suggett *et al*., 2010). It has become one of the most common, non-invasive and rapid techniques to assess the physiological state as it allows the determination of *in*-situ photosynthetic responses of seagrass without enclosing the plants in chambers that cause massive destruction to seagrass beds (Krause & Jahns, 2003; Juneau *et al*., 2005; Ralph & Gademann, 2005). Variation in PAM Fluorometer may present different characteristics in terms of approximate detection limit, measuring light colour, actinic light colour and configuration (Figueroa *et al*., 2013 and Zeng & Li, 2015). These differences may lead to inconsistencies and variations in outputs, making interpretations quite different from one equipment to another.

1.5.1 Chlorophyll fluorescence measurements

The result of chlorophyll-*a* fluorescence measurement varies in every photosynthetic organism due to the changes in biomass, chlorophyll-*a* density and the efficiency state of the organism (Suggett *et al*., 2010). The descriptions of fluorescence term used in explaining chlorophyll fluorescence are summarized in Table 1.2.

Table 1.2: The terminology used in chlorophyll fluorescence measurement from Consalvey *et al*. (2005).

	Fluorescence term	Description
Dark	F_o	Dark adapted minimum fluorescence yield
	F_m	Maximum fluorescence yield during the saturating light flash
	F_v	Variable fluorescence
	F_v/F_m	Maximum quantum yield of Photosystem II
Light condition	Ft	Light adapted minimum fluorescence yield
	F_m'	Maximum fluorescence yield in light adapted state during the saturating light flash
	ΔF	Variable fluorescence in light adapted state
	$\Delta F/F_m'$	Effective quantum yield of Photosystem II
	NPQ	Non-photochemical quenching

The photosynthetic apparatus is manipulated by three different light (Ralph & Gademann, 2005). The first light (0.15 µmol photons $m^{-2}s^{-1}$) is used to induce fluorescence emission without inducing photosynthesis. It is used to determine the amount of closed reaction centres in PSII and measuring the minimum fluorescence yield (*F* for light adapted and *Fo* for dark adapted condition) before the saturating pulse. The second light is the saturating pulse (>10,000 µmol photons $m^{-2}s^{-1}$, 0.4 - 0.8 s) which is used to close all the PSII reaction centre. It is used to measure the photosynthetic activity and resulting in higher fluorescence emission as the maximum fluorescence yield (F'_m for light adapted and F_m for dark adapted condition). The third light is called actinic light that is used to manipulate the photosynthetic apparatus in plants, mainly to induce photosynthesis (~2000 µmol photons $m^{-2}s^{-1}$).

PAM Fluorometer provides a quantitative data about PSII by measuring chlorophyll fluorescence during photosynthesis under two light condition: light-condition

and dark-condition (Belshe *et al*., 2007). When samples were dark-adapted or when there is no photon striking the antennae, the reaction centres of PSII were described as "open" and in a relax state as there is no photosynthesis taking place. At this stage the yield of fluorescence is at the minimum (F_o for dark adapted samples, and F_t for light adapted samples) as all the reaction centres are in oxidized state and ready to accept electrons as described in Figure1.1.

After samples were dark adapted for certain period of time (10 to 30 minutes) (Consalvey *et al*., 2005; Collier *et al*., 2009; den Haan *et al*., 2013), most of the reaction centres are fully opened and any photon that reached the reaction centre has the maximum chance of being utilised photochemically and not being dissipated as heat or fluorescence (Belshe *et al*., 2007; Cosgrove & Borowitzka, 2010). But when there is a short pulse of high light called saturating pulse subjected to the sample, the reaction centres of PSII begin to close and there will be an increase in fluorescence yield from minimum (F_o or F_t) to the maximum F_m (F_m for dark adapted samples and F_m' for light adapted samples) as shown in Figure 1.2 (Belshe *et al*., 2007; Consalvey *et al*., 2005, Cosgrove & Borowitzka, 2010).

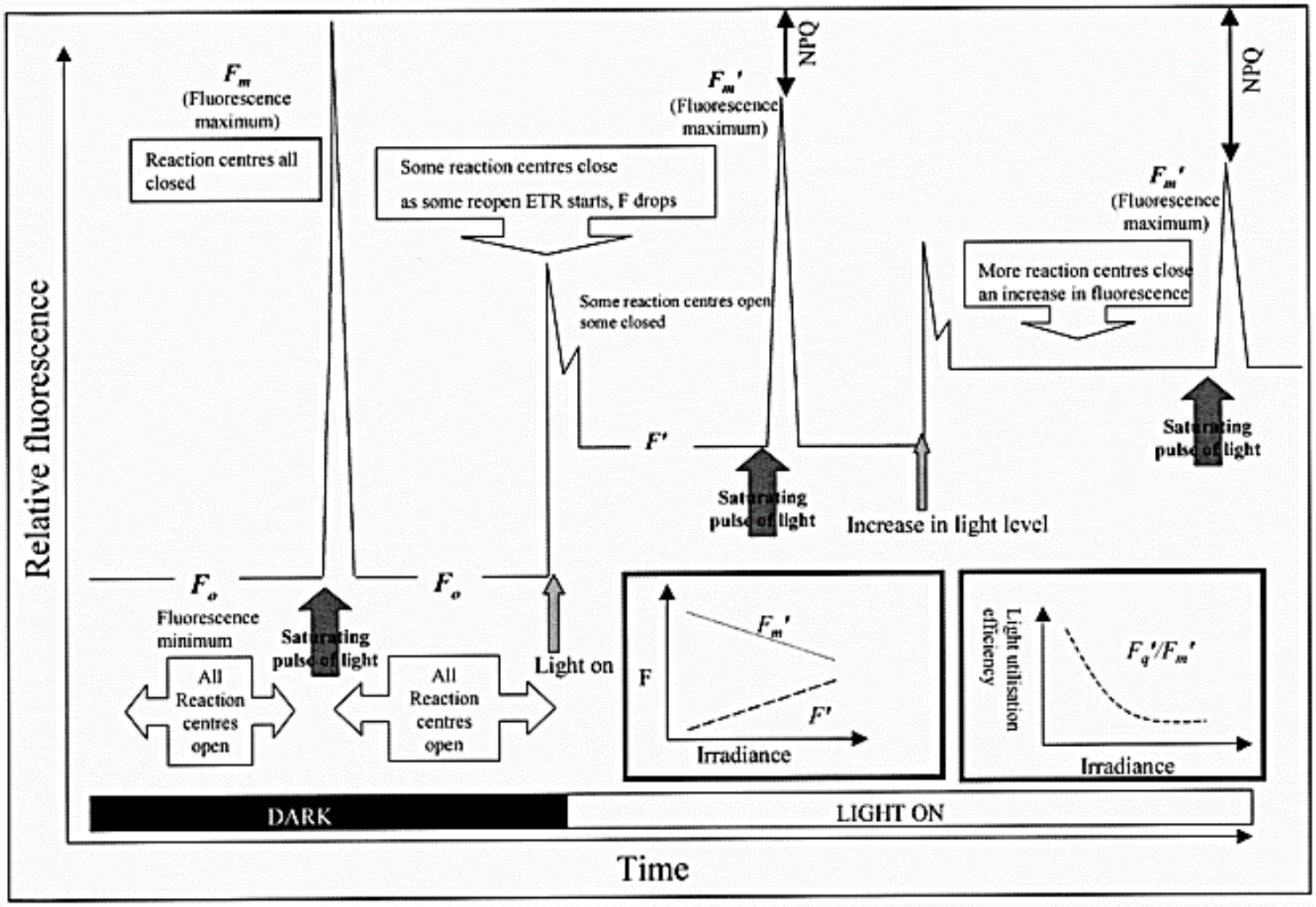

Figure 1.22: The theoretical diagram that explains the dark and light reaction of chlorophyll fluorescence output in Photosystem II from Consalvey *et al.* (2005).

The light utilisation efficiency: maximum quantum yield (F_v/F_m) and effective quantum yield ($\Delta F/F_m'$) are calculated from the measured fluorescence yield. F_v/F_m and $\Delta F/F_m'$ decrease as the light intensity increases because more reaction centres are closed. The F_v/F_m value of a healthy seagrass is 0.7 (Ralph and Short, 2002; Chesworth *et al*., 2004; Koch *et al*., 2007; Koren *et al*., 2015). The $\Delta F/F_m'$ is usually lower by comparison to the F_v/F_m due to the photoinhibition effect from non-photochemical quenching (NPQ) that reduces the light adapted yield. However, the light utilisation efficiency varies between taxa due to differences in pigment composition and cell structure (Cosgrove & Borowitzka, 2010; Consalvey *et al*., 2005; Ralph and Gademann, 2005, Beer *et al*., 2000). Decreases in light utilisation efficiency, nevertheless could also be a stress indication from the environmental parameters such as light availability (Ralph and Gademann, 2005), temperature (Campbell *et al*., 2006; Collier & Waycott, 2014), nutrients (Kilminster *et al*., 2006; Campbell *et al*., 2003) and salinity change (Marin-Guirao *et al*., 2011; Sandoval-Gil *et al*., 2012).

1.5.2 Fluorescence quenching pathway

Quenching analysis is often used to monitor the development of photochemical quenching, qP and non-photochemical quenching, NPQ (Schreiber, 2004). As noted, excited photons captured by chlorophyll *a* molecule in the reaction centre of PSII will undergo three competitive pathways; emitted as fluorescence, drive photosynthesis (photochemistry quenching; qP) and dissipated as heat by NPQ. qP is the photochemical energy utilization by charge separation at the PS II reaction centers. When all reaction centres are open, qP = 1 and when all centres are closed, qP = 0 (Schreiber *et al*., 1986). NPQ is a down-regulation quenching mechanism that diverts

excess light energy away from the PSII reaction centres to avoid photo-damage by extreme light intensities (Schreiber 2004) and can be calculated using the Stern-Volmer equation.

In dark-adapted state, qP is the maximal and NPQ is the minimal. When samples undergo dark adaptation, the photosynthetic apparatus will reach a relatively stable state with a fully oxidized state PS II acceptor, Q_A but without the trans-thylakoid proton gradient. As PS II quantum yield, qP and NPQ are correlated, when light is applied to the dark-adapted sample, PS II quantum yield will gradually decrease due to the closure of reaction centers (decrease in qP) and stimulate the heat dissipation (increase in NPQ). Hence, qP are usually used to estimates the amount of open reaction centres in PS II and the availability of PSII capture the photons (Suggett *et al*., 20100; Consalvey *et al*., 2005). However, the NPQ component is dependent on the light history of the sample and light conditions on the time of measurements were taken (Suggett *et al*., 2010). During the application of saturation pulse in dark adapted samples, the highest of fluorescence yield (F_m) will be achieved as no NPQ reaction takes place. But in light adapted samples, NPQ will happen to quench the fluorescence yield (reduce the numbers of 'open' reaction centres) which causes the maximum fluorescence yield (F_m') to be lower; thus $\Delta F/F_m'$ is lower than F_v/F_m.

1.5.3 Photosynthetic parameters determined from the Rapid Light Curves (RLCs)

RLCs are generated from PAM fluorescence with the aid of Win-Control 3.25 software (Schreiber *et al*., 1986; Schreiber, 2004) by fitting the relative electron transport rate (rETR) against the Photosynthetically Active Radiation (PAR, µmol photon m^{-2} s^{-1}) into an exponential curve (Figure 1.2). There are three important

sections in the RLCs which are: the light limited region, light-saturation point and the photoinhibition (Figure 1.3). Additional information of the photosynthetic parameters such as maximum electron transport rate ($rETR_{max}$), photosynthetic efficiency (α) and light saturation index (E_k) can be derived from RLCs. RLCs is used to asses not only the present photosynthetic activity but also the potential activity of any photosynthetic organism over a wide range of ambient light intensities. Besides this, RLCs are also able to present detailed information regarding the saturation characteristics of the electron transport rate (Consalvey *et. al.*, 2005).

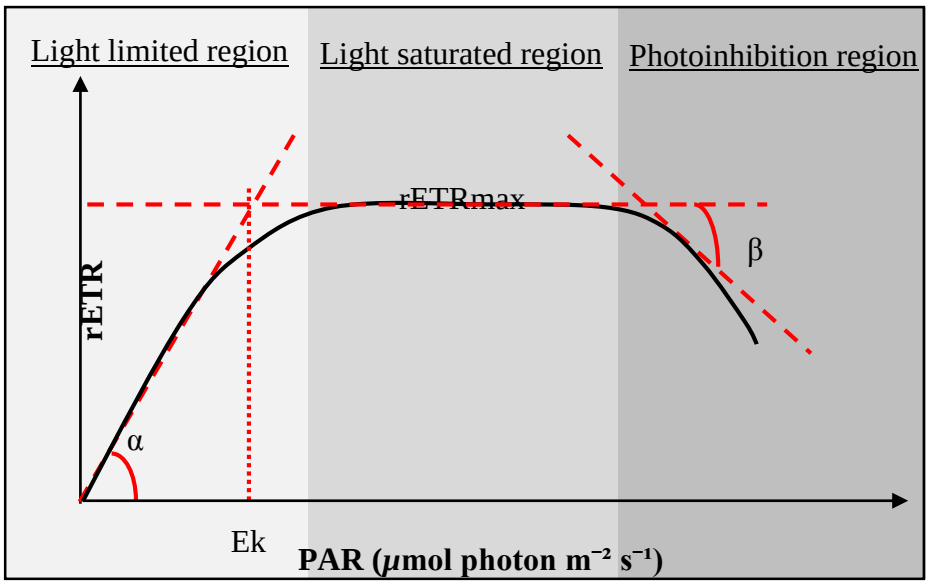

Figure 1.3: Rapid light curve adapted from Ralph & Gademann (2005).

1.6 Problem statement

In Peninsular Malaysia, Bujang *et al*., (2006), Bujang *et al*., (2012) and Bujang *et al*., (2016) summarized the known seagrasses distribution and existence sites in Malaysia. The extent of seagrass habitats and condition status around the Northern region are less reported. To date, there are six seagrass habitats reported in the northern region of Peninsular Malaysia and one of it is the study site; Pulau Korea, which is one of the crucial seagrass beds in Penang. Pulau Korea is rich in biodiversity (Yahaya, 2011; Yin & Kwang, 2016) with a significant number of coastal communities relying on it for their livelihood. However, Penang States Development Corporation had proposed to reclaim Pulau Korea. Penang Island in particular is a highly urbanised city state in Northern region of Malaysia. However, with all of the rapidly growing coastal developments adjacent to the study site which will disrupt the water quality, there have been limited studies of seagrass conditions in terms of photo-physiology nor the recent distribution are since year 2016 especially in Pulau Korea Kecil. The last updates on the distributions were reported by Nordin (2013) and Jonik (2015). In the past studies, there are more seagrass species reported in Pulau Korea which were *Halophila beccarii* Aschers, *Halophila spinulosa* Aschers, *Halophila ovata* Gaudich and *Enhalus acoroides* (Abdullah & Anscelly, 2015; Yasin & Hwai, 2008 quoted in Razalli *et al*., 2011; Hwai *et al*., 2007 quoted in Razalli *et al*., 2011). *H. ovalis* is the most common seagrass species in the tropical waters and widely covers the areas of the shallow margins present in this study, as well as in most of the seagrass meadow reported in Malaysia. However, from the past studies in 2014 and 2015 by Jonik (2015), only *H. ovalis* and *E. accoroides* were recorded in Pulau Korea. Moreover, with all the disturbances around Penang waters, there was no report on the

health status of the existing seagrass in Pulau Korea that can be used as a baseline data to predict the continuous existence in the future. The health of seagrasses can be determined by monitoring the photosynthesis performances through chlorophyll-*a* fluorescence measurement as done by many researchers (Durako, 2012; York *et al*., 2013).

Furthermore, one of the important factors in manipulating the distribution and survival of seagrasses are their capability to undergo photosynthesis effectively. Survival of seagrass is highly dependent on the photosynthesis process, which in turns influence the seagrass distribution especially those inhabiting the turbid waters as they are particularly sensitive to reduction in light availability. Penang Island in particular is a highly urbanised city state in Northern region of Malaysia. These anthropogenic event and nearby land reclamation will disrupt the water clarity and this has cause concern to the light availability for existing seagrasses in Pulau Korea. Therefore, it is important to characterise the health status of seagrass in Pulau Korea by monitoring the photosynthesis performance and the ability of *H. ovalis* to thrive in turbid waters of Penang as well as the possible seagrass limiting factor to ensure continuous existence of the seagrass population in the future. Sufficient light is important for seagrass as it controls the quality of photosynthesis to maintain positive outcome of seagrass distribution (Collier *et al.*, 2012). However, when there is a decrease in the quality or quantity of light in a particular seagrass area, the deterioration in seagrass distribution will be observed, which eventually will caused seagrasses associated fauna decreased as well. Moreover, when there is less seagrass coverage, water clarity will be reduced when there was resuspension of sediment in water column during wet season (Collier *et al.*, 2012). All of these consequences will

have high impact especially on the local communities that looking for livelihoods within the seagrass area.

Apart from exposure to high turbidity and sedimentation, seagrasses in Pulau Korea are often exposed to different light availability due to tidal changes. During low tide, seagrasses are highly vulnerable to thermal stress, desiccation and photodamage daily due to persistent exposure. Over a long period of time, these conditions may affect the abundance and photosynthetic rate of seagrass. Therefore, to understand the survival mechanism of seagrass in Pulau Korea and its overall resilience ability to recover from the light stress, a dark-recovery analysis was conducted after *H. ovalis* was exposed to two different *ex*-situ light treatment; high-light (HL) and low-light (LL). This information can be used as a baseline data for the light threshold that will not affect the seagrass condition to a critical level, especially its distribution.

1.7 Study objectives

The objectives of this thesis are as follows:

1. To provide an updated baseline data of distribution pattern, biomass and photo-physiology of *Halophila ovalis* in the intertidal zone of Pulau Korea, Penang.
2. To determine environmental parameters that influence most on the survival of seagrass as well as the adaptation of seagrass to survive under turbid water in the intertidal zone of Pulau Korea, Penang.
3. To study the effects of the different light levels on the photosynthesis and the recovery of *Halophila ovalis* in Pulau Korea.

1.8 Hypothesis

1. The distribution patterns, morphological characteristics and photo-physiology responses of *Halophila ovalis* at the intertidal zone of Pulau Korea are influenced by the spatial and temporal variations in the environmental parameters.

2. Seagrass in Pulau Korea has the ability to develop a protective mechanism to avoid excess stress and photoinhibition by having longer dark-recovery durations after exposed to high-light (HL) conditon than in low-light (LL) condition hence, enabling it to thrive in their habitat with high F_v/F_m value.

1.9 Thesis outline

This thesis examines the ecology and photo-physiology responses of seagrass at the intertidal area of Pulau Korea that affected by the variations in environmental parameters for 11 months (2016 – 2017). The responses of seagrass can be used as biological indicators in Penang waters to determine the environmental factors that have a significant effect on seagrass communities.

Chapter 2 presents the snapshot of the current status of seagrasses in Pulau Korea by monitoring the distribution and the photosynthetic performance of *Halophila* ovalis (dominant seagrass) in Pulau Korea. This chapter highlighted some of the reasons why persistent monitoring of seagrass and conservation should be done in Pulau Korea.

Chapter 3 investigates the effects of high-light (HL) and low-light (LL) exposure on the photosynthesis performances and recovery of *H. ovalis*. The acclimation mechanisms in *H. ovalis* enabled them to tolerate and/or acclimatize to extended periods of altered light regime conditions. In addition, this chapter explored the resilience mechanism of *H. ovalis* through dark-recovery periods (10, 15, 30 and 60 minutes) after HL and LL exposures. This chapter allows us to have a better understanding of the photosynthetic strategy of *H. ovalis* in Pulau Korea which allows it to survive under harsh water condition in Penang.

Chapter 4 and Chapter 5 discuss and summarize the findings in both chapter 2 and 3 by relating the study outcome of both chapters. Data obtained in this study can used as a baseline data for an effective seagrass monitoring programme and conservations.

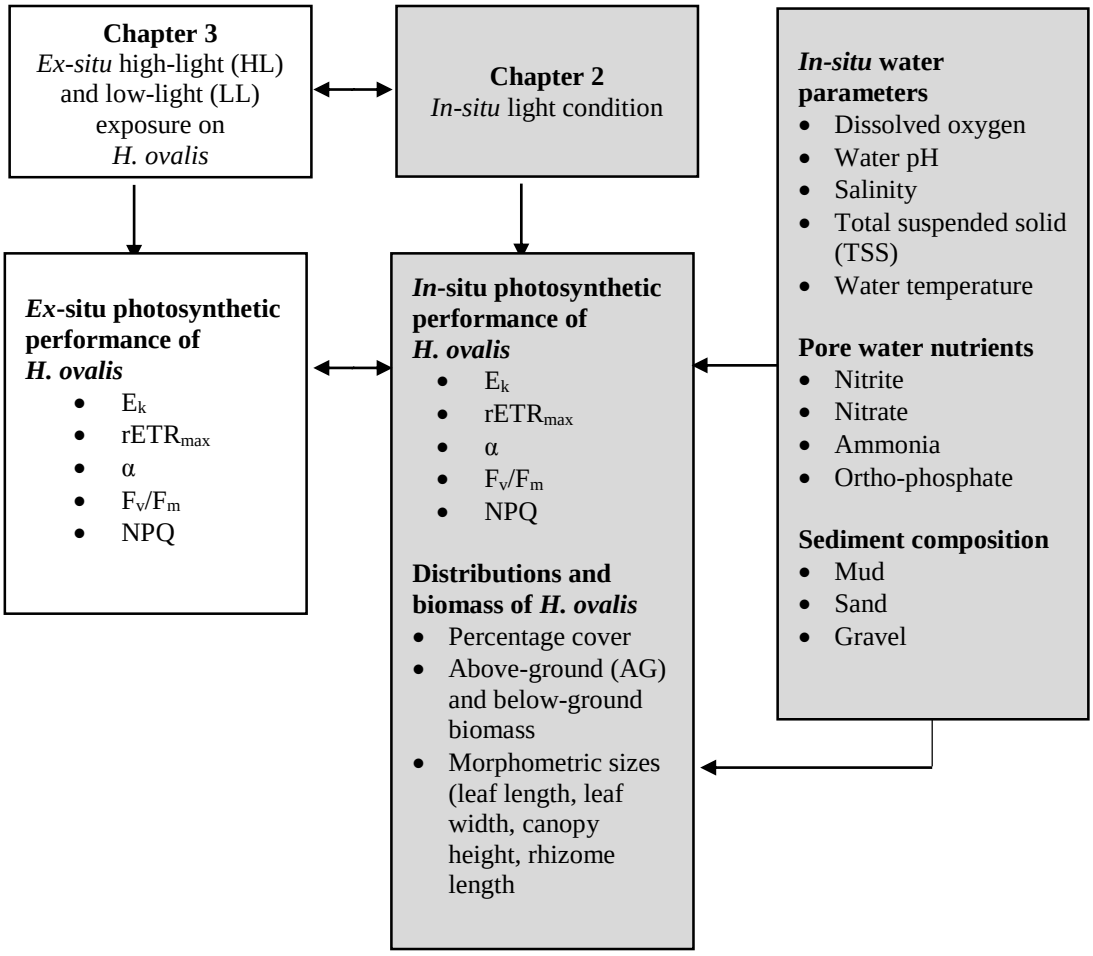

Figure 1.4: Thesis outline according to chapters.