# PHOTOSYNTHETHIC PARAMETER ASSESSMENT OF CORAL ZOOXANTHELLAE OF *Acropora aspera* AND *Favites abdita* USING MAXI I-PAM SYSTEM IN HERON ISLAND, GREAT BARRIER REEF, AUSTRALIA

**Undergraduates Thesis**


**By:**

**SIHAM AFATTA**

**K2D001342**




**MARINE SCIENCE STUDY PROGRAM**

**FACULTY OF FISHERIES AND MARINE SCIENCE**

**DIPONEGORO UNIVERSITY**

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**Undergraduates Thesis as a Requisite**
**To Acquire the Undergraduates Degree**
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**APPROVAL**

| | | |
|---|---|---|
| Research Title | : | Photosynthetic Parameter Assessment of Coral Zooxanthellae of *Acropora aspera* and *Favites abdita* Using MAXI I-PAM System in Heron Island, Great Barrier Reef, Australia. |
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| Department / Study Program | : | Marine Science / Marine Science |

This an official English translation of an undergraduates thesis report titled:
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which had accomplished a thesis viva with the defence committee in:
April 24th 2006.

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**RESEARCH AUTHENTICITY STATEMENT**

Hereby I, Siham Afatta, declare that this scientific research / undergraduates thesis, which is an independent work of research, has never been proposed in any fulfillment guideline to acquire a strata one ($S_1$) undergraduates degree at Diponegoro University or any other educational institutions.

All of the information included in this scientific research which were obtained from other writers, yet publicized or not publicized, has been awarded by citing the source writer's name accurately and as a writer I would be fully responsible for all the contents inside this scientific research / undergraduates thesis.

Semarang, February 2007

Writer,

Siham Afatta

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## SUMMARY

**Siham Afatta. K2D001342.** Photosynthetic Parameter Assessment on Coral Zooxanthellae of *Acropora aspera* and *Favites abdita* using MAXI I-PAM System in Heron Island, Great Barrier Reef, Australia.
**(Supervisor: Ir. Wisnu Widjatmoko, MSc. and Dr. Ir. Ambaryanto, MSc.).**

Zooxanthellae, which reside inside the coral host, have been an essential component in coral ecosystem studies. While residing inside their host, zooxanthellae photosynthesize provide energy and nutrients to coral. Objectives are to observe the impact of increasing light treatment to the coral by studying correlation between photosynthetic parameters and the cell condition of zooxanthellae.

Materials used for this research were coral samples of *Acropora aspera* and *Favites abdita,* acquired from shallow waters (<2m) in the lagoons of Heron Island, Australia. This research was conducted from the first until the ninth of August 2005. Sample analysis was conducted in Director's Laboratory at Heron Island Research Station (HIRS), Queensland University, Australia. Samples were obtained with Stratified Random Sampling method. Zooxanthellae parameters measured were cell size, cell density, and chlorophyll concentration per cell. Photosynthetic parameters measured from coral samples were values of Effective PSII Quantum Yield (Y(II)) and Non-Photochemical Quenching (NPQ/4) by using MAXI-Imaging-Pulsed Amplitude Modulation (I-PAM) System with PAR treatments ranging from 0 to 1251 µmol quanta $m^{-2}$ $s^{-1}$. I-PAM data were measured using ImagingWin version 1.01 software (Heinz Walz GmbH, Germany), graphs and charts analysis were used to observe the correlation between zooxanthellae and photosynthetic parameters.

Results for three colonies of *A. aspera* and *F. abdita* had shown fluctuation tendency in Y (II) and NPQ/4 values is a decrement of Y(II) values parallel with the increment of NPQ/4 values; a similar condition was also observed in inter-species comparison. Three colonies of *A. aspera* show that the value of cell density tends to be proportional to the Y(II) value resistance and NPQ/4 value attainment. Similar results, with the exception of PAR limit reaching 461 µmol quanta $m^{-2}$ $s^{-1}$, was also occurred in three colonies of *F. abdita*. During treatments from 0 until 1251 µmol quanta $m^{-2}$ $s^{-1}$ PAR, a two species comparison of *A. aspera* and *F. abdita* show that Y(II) value resistance and NPQ/4 value attainment in *A. aspera* tends to be lower than *F. abdita;* which was proportional to the increase of cell size and also chlorophyll concentration of zooxanthellae.



***Keywords*** **:** Photosynthesis, Zooxanthellae, Coral, *Acropora aspera, Favites abdita,* Pulsed Amplitude Modulation, Y(II), NPQ/4.

## FOREWORD

Alhamdulillah, praise be to Allah, who is always giving guidance and insights to mankind. Thus, this research report could be well accomplished.

The writer also likes to express thanks to:

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3. Prof. Ove Hoegh-Guldberg who had shared us a valuable study experience and also assisted during this research.
4. All of our friends, partners, and institutions who has supported this research.

Any critics and suggestion will be expected by the writer as it will contribute to our excellence of this research and upcoming. We hope that this research will be useful to mankind, animals, plants and the environment. Amin.

Semarang, Maret 2006

Writer

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# CHAPTER I

# INTRODUCTION

## 1.1 Backgrounds

The ocean's primary productivity relies on organic nutrient resources, formed by inorganic materials using sunlight, from photosynthesis processes by marine organisms (Campbell, 1990). Resembling to the high terrestrial plant, marine plants cells also contained photosystem II components as one of the photosynthesis apparatus (Hoegh–Guldberg, 1999) which is highly sensitive to light as well as processing it (Campbell, 1990).

As a mutualistic symbiont, zooxanthellae give their energy and nutrients to their host by allocating up to 95% of their photosynthesis products (Muscatine, 1990). As a photosynthetic plant, zooxanthellae has a role in processing sunlight radiation efficiently and is able to securely divert the over-excited photon energy (in heat) (Demmig-Adams, 1996 *in* Brown *et al.*, 2002).

High intensities of sunlight can cause disturbances in light harvesting efficiency during the photochemical process in photosystem II (Jones *et al.*, 1998) and triggers the quenching process of heat reduction from over-excitation energy (Brown, 1999 *in* Brown, 2002). Continuity in light stresses can also cause damage to the zooxanthellae cell component (pigments and chloroplast, Salih *et al.,*1999) until zooxanthellae is expelled from coral tissue; this is indicated with a pale appearance of coral colour, generally known as coral bleaching (Hoegh-Guldberg, 1999).

Consequently, stresses on coral caused by high light intensity can be indicated with disturbance of light harvesting efficiency in photochemical

reactions in photosystem II (Effective Photosystem II Quantum Yield) and in occurrence of the light protection mechanism (Non-Photochemical Quenching).

Nowadays, mass coral bleaching frequency is increasing which is commonly caused by temperature rise and high light intensity from global warming (Hoegh-Guldberg, 1999). An advanced study relating to zooxanthellae photosynthesis mechanism would give essential information in the efforts to understand coral species' characteristics under high light intensities.

## 1.2 Scope of the Research

Mutualistic symbiosis between coral hosts and zooxanthellae is an important factor in the coral ecosystem as zooxanthellae loss would lead to coral death. The symbiosis is very sensitive to anthropogenic impacts (Veron, 2000) and research has also shown that environmental changes have recently increased the frequency of global coral bleaching (Wellington *et al*., 2001 *in* West and Salm, 2003). There are many environmental factors that can cause coral bleaching (Brown and Howard, 1985; Jones, Kildea and Hoegh-Guldberg, 1999; Hoegh-Guldberg and Jones, 2001), but generally, a mass coral bleaching is caused by threshold-exceeding temperature rise and increasing sunlight intensity (Hoegh-Guldberg, 1999).

Photosynthetic observations of terrestrial plants have shown that photosystem II is a light and temperature-sensitive component. Where various researches on coral bleaching, assessed in photosystem II, commonly observed chlorophyll *a* component (Iglesias-Prieto, 1995; Jones *et al*., 1998; Jones and Hoegh-Guldberg, 2001; Ralph *et al*., 2001).

The negative impacts of increasing light intensity and temperature were related to a reduction of photosynthetic efficiency within photosystem II that will lead to coral bleaching. Whereas the Photosynthetically Active Radiation (PAR) is the main factor that causes bleaching by high intensity of light.

Therefore, this research was conducted to observe the photosynthetic mechanism in the photosystem II component of zooxanthellae cell chlorophyll throughout the increasing saturated light treatment, by observing coral fluorescence using Pulse Amplitude Modulation (PAM). The research flowchart showed in Figure 1.

## 1.3 Objectives of the Research

The objective of this research is to acquire the values of Effective PS II Quantum Yield and Non-Photochemical Quenching related to cell density per coral colony, chlorophyll concentration per cell, and cell sizes of zooxanthellae that reside within *Acropora aspera* and *Favites abdita* coral species.

## 1.4 Benefits of the Research

We expect that the research will be an essential source of information in understanding the mechanism of zooxanthellae photosynthesis related to coral bleaching phenomena, which eventually will be useful in conservation and management of global coral reef to cope with global warming stresses ahead.

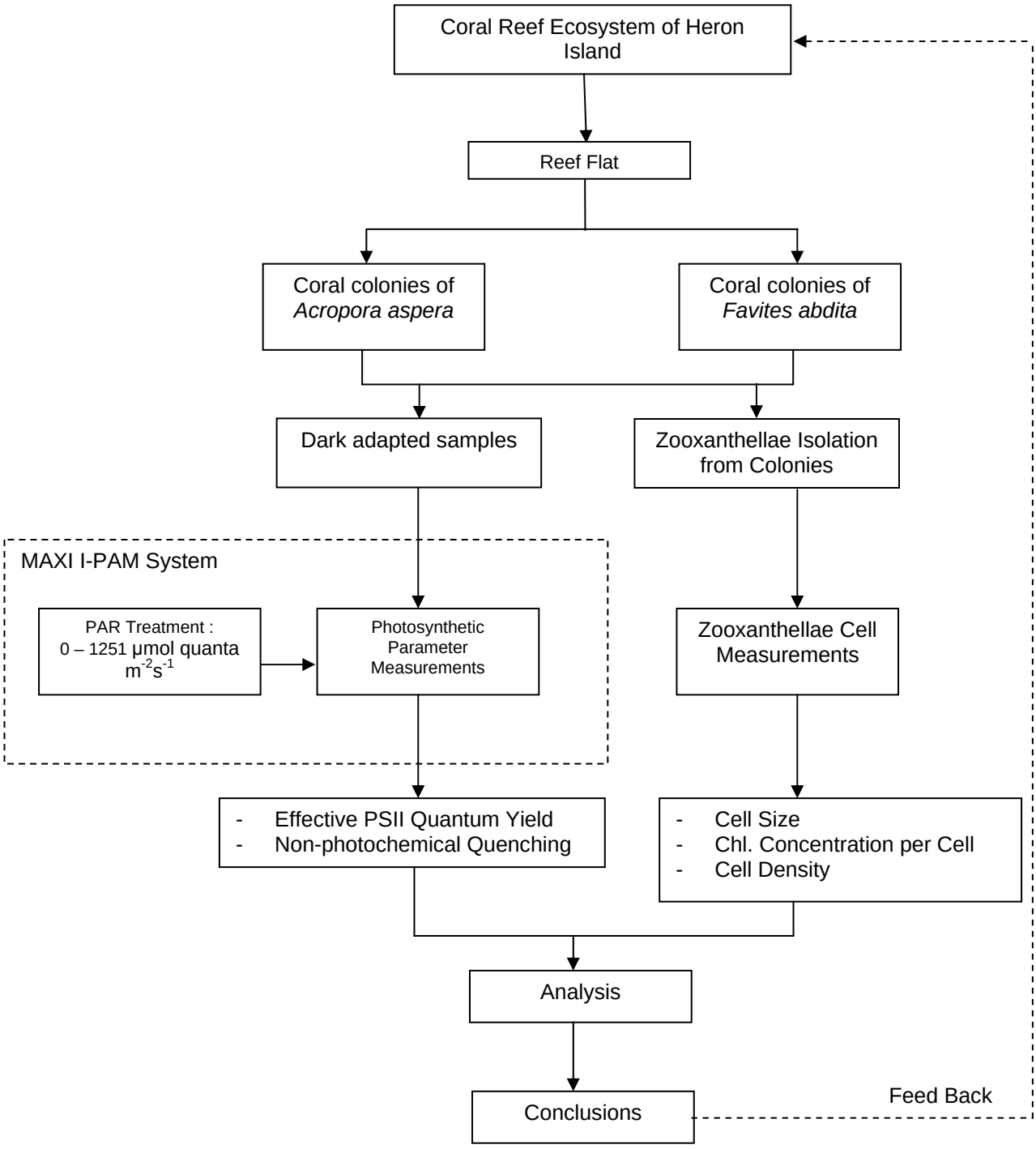


Figure 1. Research flowchart

# CHAPTER II

# LITERATURE STUDIES

## 2.1. Coral Reef

### 2.1.1. Coral Reef Ecosystem

Coral reefs are organisms that live in the bed of tropical and subtropical shallow waters. Coral reef ecosystem, mostly consist of anthozhoan corals and the scleractinian class which are members of hermatypic corals, species of corals that are capable of building a structure or a skeleton of calcium carbonate (Vaughan and Wells, 1943 *in* Supriharyono, 2000). A relatively strong calcite structure ($CaCO_3$) enables corals to withstand the impact of wave force. While the association of the organisms which resides in the ecosystem, besides scleractinian corals, is the algae which several among them also restrain calcium carbonate (Dawes, 1981 *in* Supriharyono, 1990). Suharsono (1996) stated that the formation of coral reef begins with the organisms bonding, which restrain calcium carbonate, in a complex and slow process.

### 2.1.2. Coral Biology

Coral is a primitive tube-like animal with a mouth on its top that is also utilised as an anus (Suharsono, 1996). In Veron (2000) it was also mentioned that corals basically are anemone-like animals, however, corals are capable of secreting skeleton / calcareous structure, by living in colonies and by living solitary.

Inside the polyp tissue is the coelenteron, a sac-like body cavity which has single openings to the outside. The coelenteron of one polyp is linked to

those of adjacent polyps by tubes through which water circulates and nutrients are transported. The polyp mouth opens through the pharynx towards body cavity, which is capable of extend away from the skeleton to catch food alongside with the tentacles. Coelenteron were consists of mesenteries, which give space for digestion, photosynthesis and respiration, and reproductive organs (Veron, 2000). A generalized polyp and corallite structure is illustrated in Figure 2.

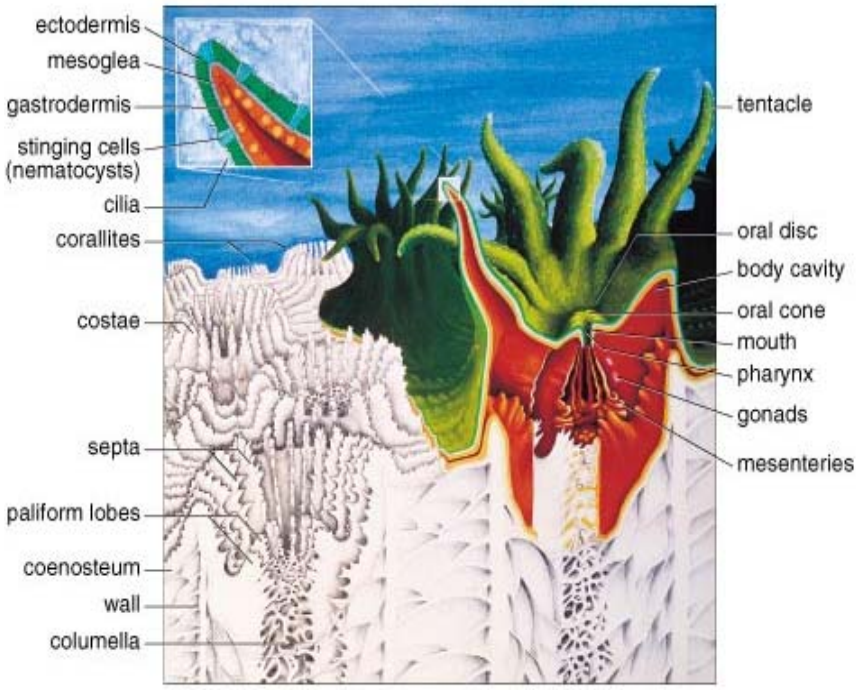


Figure 2. General structure of polyp and corallite (Veron,2000)

Each coral polyp consists of three layers of tissue, which are ectoderma as an outer tissue, endoderma (inner tissue) which contains algae cell (Zooxanthellae) and mesoglea as a jelly-like tissue layer between ectoderma and endoderma (Suharsono, 1996). In the ectoderma are many glandule cells which contain mucus, and knidoblast cells which contain nematocysts cells. The nematocyst cells is stinging cell that is utilized to catch food and for self

defence. While the mucus cells were utilized as a mucus producer that helps in catching food and as protection from settling sediments (Suharsono, 1996).

According to Nybakken (1992) coral growth rate depends on its species, colony age, and reef habitat, and the growth form also varies depends on habitat conditions. A thinner coral species would be found on deeper waters rather that shallow, where large waves tend to result in a shorter, blunt growth of branching corals, and the current would affect the growth in a certain direction.

### 2.1.3. Species Characteristics of *Acropora aspera*

Colonies of *Acropora aspera* (Dana 1846) are corymbose clumps or tables with thick branches of highly variable length depending on exposure to wave action. Axial corallites are small but distinct. Radial corallites are of two sizes, crowded, and have prominent lower lips giving a scale-like appearance. Colors are usually pale blue-grey, green or cream, sometimes bright blue. Habitats are reef flats and shallow lagoons, also exposed upper reef slopes and deep water. Species are sometimes common in several places. Mentioned below is the taxonomy hierarchy of *Acropora aspera* (Veron 2000):

Kingdom : Animal

Phylum : Coelenterata

Subphylum : Cnidaria

Class : Anthozoa

Order : Scleractinia

Family : Acroporiidae

Species : *Acropora aspera*

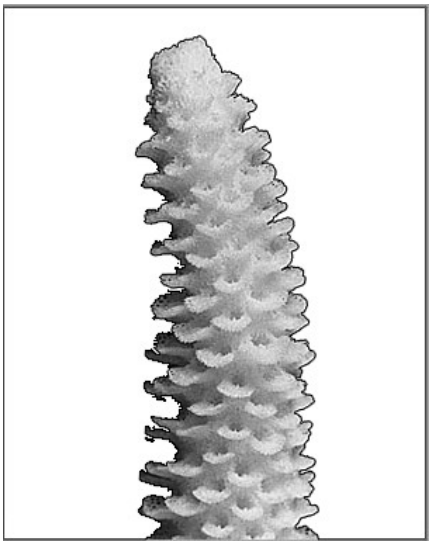

Figure 3. Skeletal structure of *A. aspera* (Veron, 2002)

### 2.1.4. Species Characteristics of *Favites abdita*

Colonies of *Favites abdita (*Ellis and Solander, 1786) are massive, either rounded or hillocky and sometimes over one meter across. Corallites are rounded, with thick walls. Septa are straight, with exert teeth. Colors are dark in turbid environments, otherwise pale brown with brown or green oral discs. Its abundance is common in most reef environments. Mentioned below is the taxonomy hierarchy of *Favites abdita* (Veron 2000) :

Kingdom : Animal

Phylum : Coelenterata

Subphylum : Cnidaria

Class : Anthozoa

Order : Scleractinia

Family : Faviidae

Species : *Favites abdita*

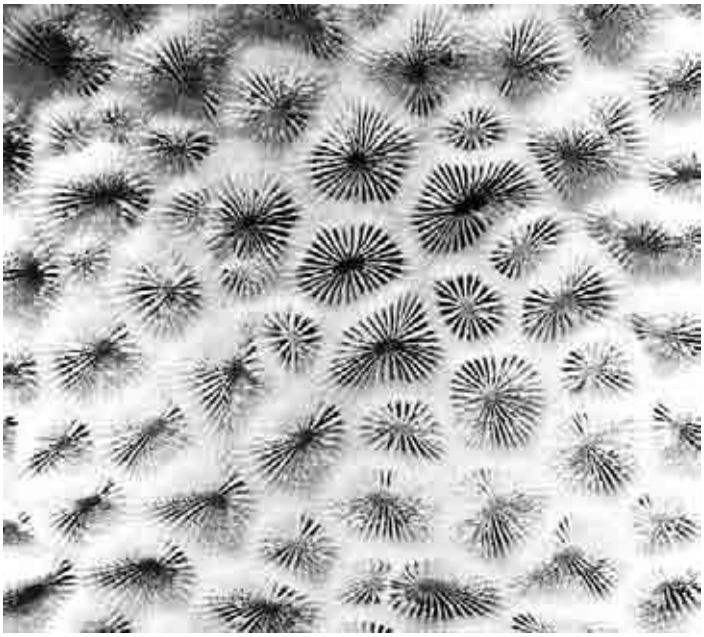

Figure 4.Skeletal structure of *F. abdita* (Veron, 2002)

## 2.2. Zooxanthellae

### 2.2.1. Histology and Physiology

Zooxanthellae is an intracellular organism, categorized as a single pandemic species of *Symbiodinium microcardiaticum* (Trench, 1979 *in* Hoegh-Guldberg, 1999) which usually found on the membrane-bound vacuole inside the host cell, with the exception of giant clams (Norton and Jones, 1992; Norton *et al.*, 1992 *in* Hoegh-Guldberg, 1999). Based on research of Trench (1979); Schoenberg and Trench (1980*a*, 1980*b*) and Rowan and Powers (1991, 1992) *in* Hoegh-Guldberg, (1999); it has been revealed that in the species *Symbiodinium microadriaticum* is a high diversity group of organisms which probably consists of hundreds of taxa and in each host may reside two or three different taxas. LaJeunesse (2002) in his research at Heron Island, discovered that based on rDNA structure analyst of species *Symbiodinium* spp., from 86

coral species, there are four clades (A, B, C, and D) in which scope 23 sub-clade found.

### 2.2.2. Symbiosis Between Coral and Zooxanthellae

#### 2.2.2.1. Zooxanthellae's Role in Coral

Corals, as a major builder of a reef, were are in symbiosis with 'zooxanthellae', a single-cell alga that resides within the endodermic layer (Figure 5). Zooxanthellae photosynthesize while residing inside their coral host which secrete calcium carbonate all year long. Organic materials from the photosynthesis were excreted partially into the polyp intestine as a food source (Suharsono, 1996), which consists of sugar and amino acid, while corals contribute in essential plant nutrition to zooxanthellae (ammonia and phosphate) from its metabolic waste (Trench, 1979 *in* Hoegh-Guldberg, 1999).

#### 2.2.2.2. Coral-Zooxanthellae Symbiotic Stability

Zooxanthellae's survival ability does not definitively depend on its coral host (Suharsono and Brown, 1992). Zooxanthellae which are expelled from their host, sometimes remain in good condition (Bhagooli and Hidaka, 2004 *in* Ralph *et al.,* 2005) and sometimes actively photosynthesize (Ralph *et al.,* 2001 *in* Ralph *et al.,* 2005). Corals, as a host, depend a lot on zooxanthellae photosynthesis products for tissue and body growth (Hoegh-Guldberg, 1999).

Light is the main factor for photosynthesis in zooxanthellae cell (Chalker *et al.,* 1988 *in* Hoegh-Guldberg, 1999). However, high intensities of lights can also disturb photosynthesis reactions, later causing zooxanthellae to be expelled by its host (Salih *et al.,* 1998). Lesser (1997) assumed that

zooxanthellae expulsion by its host was initiated by oxygen toxic production in zooxanthellae cells and in the host's cells causing cell damage.

High temperature levels (32-34$^{o}$C) can also disturb zooxanthellae photosynthesis process as subsequently interrupting the electron transport from $H_2O$ molecule fractions in light processing (Iglesias-Prieto, 1995). Other factors concerning coral-zooxanthellae symbiosis disturbance are low salinity, UV radiation, Bacterial infection and heavy metals (Hoegh-Guldberg and Smith, 1989; Lewis, 1995; Lesser *et al.,* 1990; Kushmaro *et al.,* 1996; Miller *et al.,* 1992 *in* Salih *et al.,* 1998).

#### 2.2.2.3. Coral Bleaching

Coral bleaching is an indicator for coral-zooxanthellae symbiotic disturbances caused by release of zooxanthellae from its coral host or by the decrease of chlorophyll pigment, usually noticeable by coral tissue colour change (Hoegh-Guldberg, 1999). Coral bleaching is a stress response which usually occurs in the field or in the laboratory due to different low and high temperature treatment, high light, salinity change, or by other physical and chemical pressures (Buddemeier *et al.,* 2004).

## 2.3. Photosynthesis

#### 2.2.2.1. Photosynthesis In Plant Cells

Photosynthesis, as stated by Campbell (1990), means a process that occurs inside the chloroplast organelle (Figure 6.) inside plant cells which captures sunlight and converts the light energy into chemical energy, which is

stored as bonds of sugars and other inorganic molecules made from carbon dioxide and water.

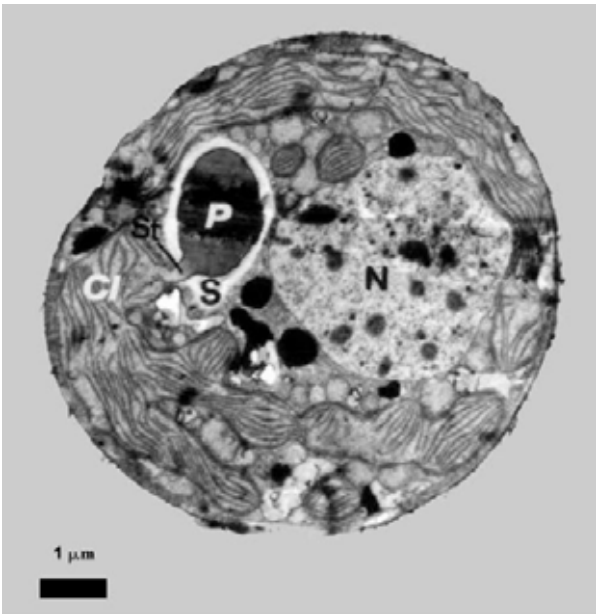


Figure 5. Symbiotic algae (zooxanthellae) from reef-building coral. P: Pyrenoid, N: Nucleus, Cl: Chloroplast, S: Starch (Photo: Takabayashi *in* Hoegh-Guldberg, 1999).

Inside the chloroplast (Figure 7), chlorophyll pigment in the thylakoid membrane will capture sunlight for synthesizing food molecules. Thylakoid membranes take part in the conversion of light energy into chemical energy phase; while its chemical energy will be used in the conversion phase of $CO_2$ into sugar which is utilizes stroma (Campbell, 1990).

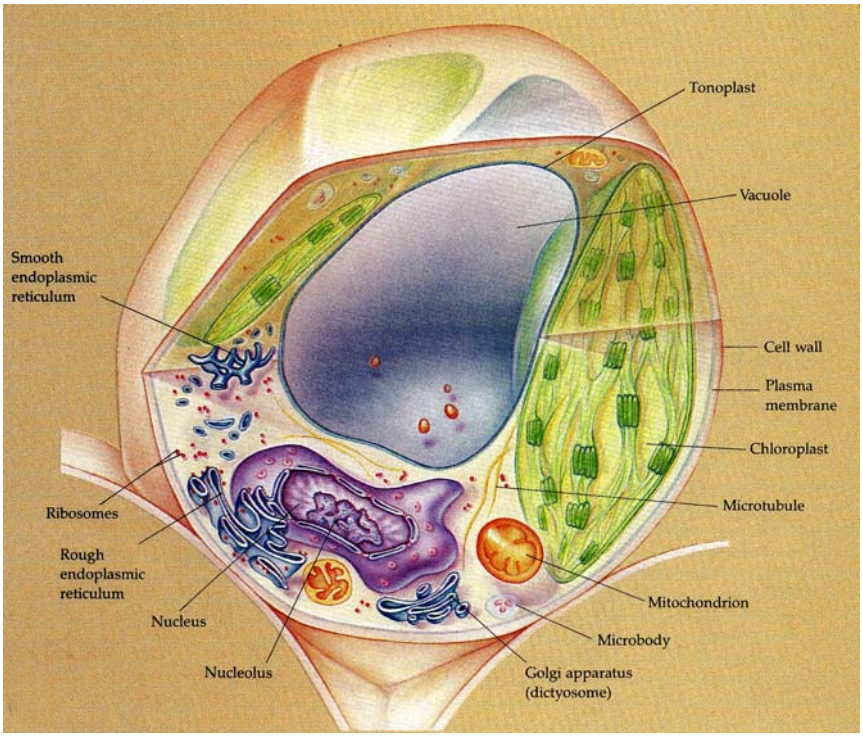


Figure 6. A generalized scheme of a plant cell (Campbell, 1990)

Campbell (1990) also mentioned the reaction of photosynthesis:

$6CO_2 + 12H_2O + \text{Light Energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$

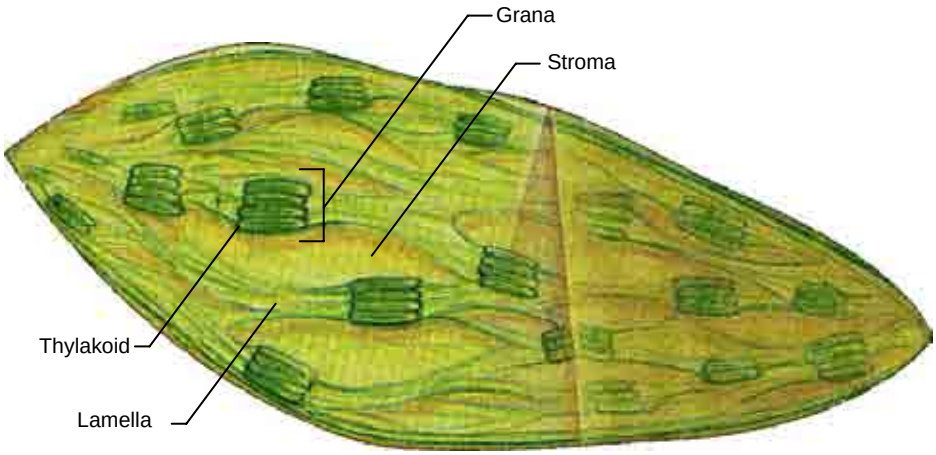


Figure 7. Chloroplast organelle of a plant cell (reconstructed from Campbell, 1990)

### 2.2.2.2. Light Harvesting in Photosynthesis

Light's nature as a particle that is usually expressed with a statement that light irradiates as a photon molecule (mole photon) or quanta, which is a discrete package of energy, whereas each one package originates a certain wavelength (Lakitan, 2001). As the light reaches at a matter (a cell organelle), it would possibly be diverged, transmitted or absorbed by a substance that receives visible light which is called a pigment (Campbell, 1990)

During visible light absorbance, a photon has caused an excitation of a chlorophyll molecule from its ground state, as the photon forces an electron towards a higher energy potential orbital. Consequently, the excited electron would return to its former orbital (ground state), releasing its energy by heat and fluorescence (Figure 8) (Campbell, 1990).

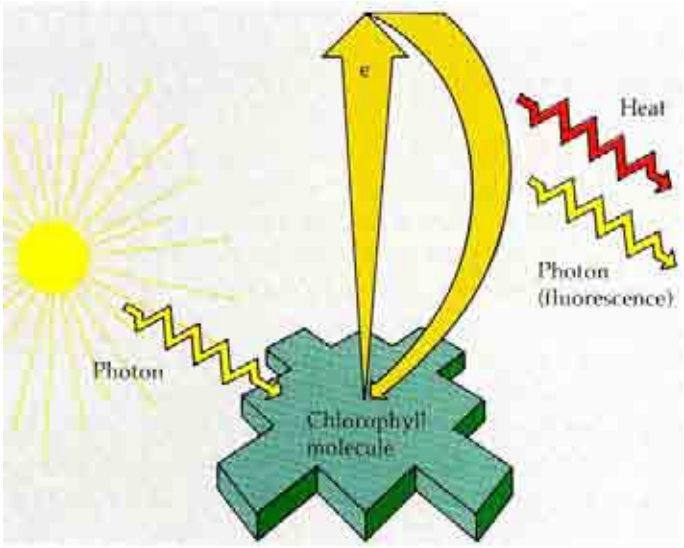


Figure 8. The interaction of a photon with a chlorophyll molecule (Campbell, 1990).

Chlorophyll *a* is an essential pigment on photosynthesis since it is directly involved in the light reactions, which converting light energy into chemical energy. Other pigments which only absorb light and transferring the

energy to chlorophyll *a* (reaction centre), such as chlorophyll *b* and carotenoid pigment (Campbell, 1990). The range of wavelengths which is absorbed for photosynthesis is approximately between 350 – 750 nm (Lakitan, 2001), where these lights are captured collectively by the molecules of chlorophyll *a,* chlorophyll *b* and carotenoid, absorbing the photons and transferring in among the molecules until it reaches the reaction centre (Figure 9) (Campbell, 1990).

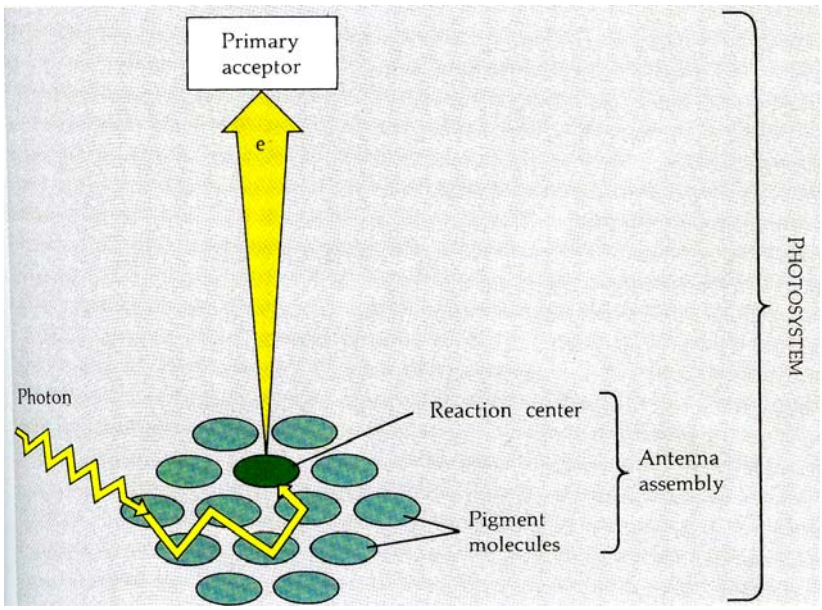


Figure 9. Photons captured, absorbed, and transferred to the reaction centre (Campbell, 1990)

### 2.2.3. Photosystem I and II

Photosystem is a light harvesting unit in the thylakoid membrane which consists of complex antenna (light capturing pigments), chlorophyll *a* reaction centre and primer electron acceptor. Each photosystem has an antenna that assembles from a few hundred of pigment molecules, including a number of chlorophyll and carotenoid molecules. Two types of photosystems that has been found inside the thylakoid membrane (Campbell, 1990) which are:

1. Photosystem I (PS I), which has molecules of chlorophyll *a* on its reaction centre, known as P700, which absorbs wavelengths approximately 700 nm.
2. Photosystem II (PS II), which has molecules of chlorophyll *a* on its reaction centre, known as P680, which absorbs wavelengths ranging from 350 - 680 nm.

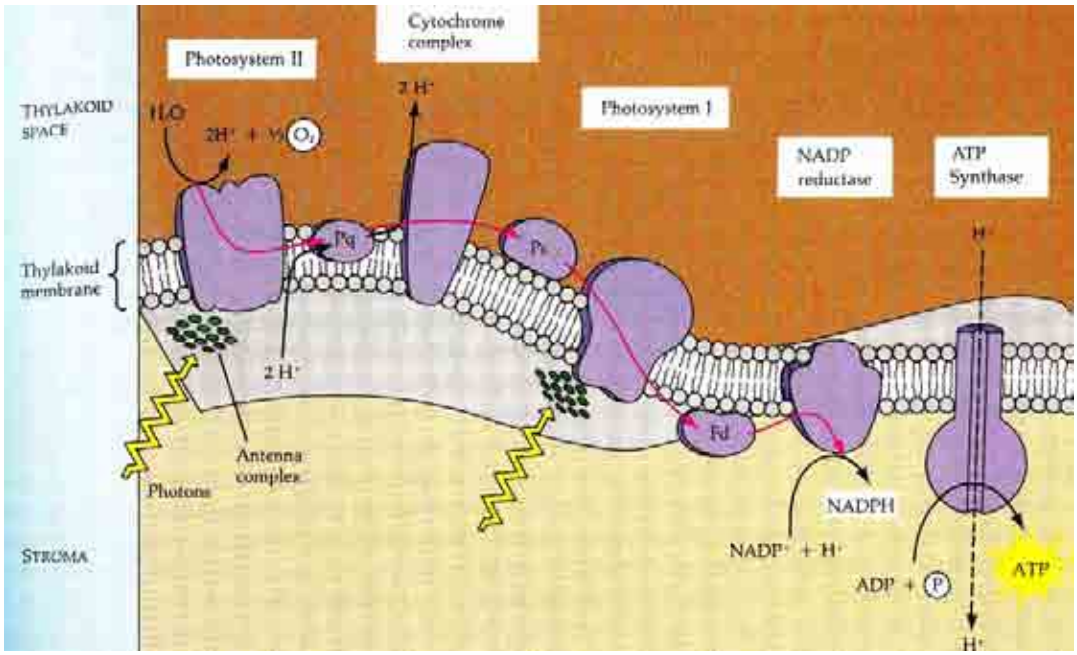


Figure 10. Light harvesting by photosystem in the thylakoid (Campbell, 1990)

## 2.4. The Impact of High Light Intensity on Coral

Based on plant research from Schreiber (1997) stated that a high intensity of 1000 µmol quanta $m^{-2}$ $s^{-1}$ as a stationary light is relevant of a full sunlight exposure. In treatments on Zooxanthellae, Lewis (1995) *in* Salih *et al.* (2005) concludes that in coral symbiotic algae, high visible light intensity could cause coral bleaching. Research done by Salih *et al.* (2005) were also shown that a stress response by symbiotic dinoflagellates (zooxanthellae) may be generated

by light intensity of 800-850 µmol quanta $m^{-2}$ $s^{-1}$ with an indication of PS II and cell morphological damages.

Research done by Hoegh-Guldberg and Smith (1989) *in* Hoegh-Guldberg and Jones (2001), on coral species of Stylhopora *pistillata*, had shown that a high light elevation itself was able to cause the lost of symbiotic algae (coral bleaching) caused by the mechanism of photoinhibition in photosynthesis.

## 2.5. Chlorophyll Fluorescence as an Energy Conversion Indicator of Photosynthesis Fluorescence

Schreiber (1997) mentioned that there are five functional levels in photosynthesis process:

1. Light absorption and energy transfer at the level of the antenna pigments
2. Primary light reactions (photochemical energy conversion)
3. Electron transport via membrane bound carriers
4. Coupled trans-membrane proton transport and ATP-synthesis
5. Enzymatic dark reactions in the stroma (CO2-assimilation)

In principle, information at all of these levels can be gained by chlorophyll fluorescence measurements and, most importantly, this can be done in a non-invasive way, i.e. without disturbing the natural course of photosynthesis (Schreiber 1997).

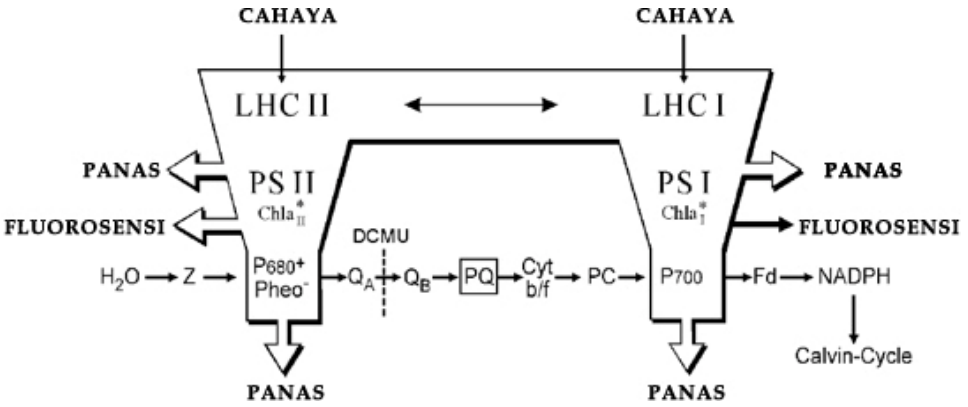


Figure 11. Schematic view of primary energy conversion and primary electron transport in photosynthesis. LHC, light harvesting pigment-protein complex; Pheo, pheophytin; DCMU, PSII inhibitor (diuron); PQ, plastoquinone; PC, plastocyanin (Schreiber, 1997)

The outstanding indicator function of chlorophyll fluorescence results from the fact that fluorescence originates from the same excited states which alternatively can be used for photosynthetic energy conversion or also be non-radiatively dissipated into heat. (Schreiber, 1997).

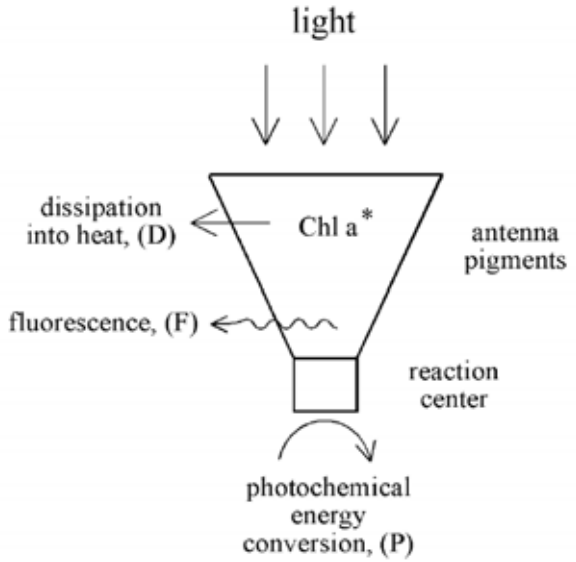


Figure 12. Schematic view of light conversion in photosystem II (Schreiber,1997).

Under *in vivo* conditions, fluorescence changes originate almost exclusively from chlorophyll *a* localized in PS II (including its antenna pigments). Therefore, chlorophyll fluorescence *in vivo* can give immediate information on the use and dissipation of excitation energy in PS II only. (Schreiber, 1997).

# CHAPTER III

# MATERIALS AND METHODS

## 3.1. Time and Location of Research

The research was conducted in 1st- 9th August, 2005. The samples were observed in Director's Laboratory of Heron Island Research Station, University Of Queensland, Australia.

Heron Island lies in a 31 $km^2$ lagoon in the Capricorn Bunker region of Great Barrier Reef. The reef of Heron Island is considered as a habitat for 900 of 1500 fish species, and 72% species of coral that found throughout Great Barrier Reef, Australia. An approximately 17 ha of the island vegetation is a subtropical forest. The location map of the research is available in Appendix 1.

## 3.2. Materials and Tools

### 3.2.1. Materials

The materials used in this research are two coral species of *Acropora aspera* (branching coral), and *Favites abdita* (massive coral) that are taken from Heron Island's reef flat, in shallow water (Depth < 2 m).

Figure 13. Coral sample, *Acropora aspera*

Figure 14. Coral sample, *Favites abdita*

### 3.2.2. Tools

The research requires tools as listed bellow:

Table 1. Tools required in the research

| No. | Name of tool | Accuracy | Function |
|---|---|---|---|
| 1. | Snorkelling set | - | To facilitate the underwater movement while taking samples |
| 2. | Pliers | - | To take coral samples |
| 3. | Plastic bag | - | To carry the samples |
| 4. | Labels | - | To give labels on samples |
| 5. | Water picker | - | To take off tissue from coral skeleton |
| 6. | Filtered seawater, 0.45 µm Whatman filter | - | Resuspension medium of tissue during zooxanthellae isolation |
| 7. | Centrifuge | - | To separate zooxanthellae cell from resuspended coral tissue(4730 x g) |
| 8. | Centrifuge tubes | 50 ml | To place resuspended samples during the homogenizing |

| | | | |
|---|---|---|---|
| 9. | Binocular Microscope | 100 x zoom in | To observe Zooxanthellae cell |
| 10. | Haemocytometer | - | As a base during zooxanthellae cells counting under the microscope |
| 11. | Glass slide | - | As a base during zooxanthellae cell size measurement under the microscope |
| 12. | Graticule eye piece | Line scale 10 ± | To measure the zooxanthellae cells sizes under the microscope |
| 13. | Emerson Oil | - | To prevent contact of objective lens with glass slide |
| 14. | Hand counter | - | To note down the cell counts |
| 15. | Measuring glass | 250 ml | To measure resuspended solution of isolated tissue |
| 16. | Spectrophotometer Ultrospec III | Wavelength, 630, 663 nm | To measure the absorbance of resuspended solution |
| 17. | MAXI Imaging-PAM | - | To measure Y(II) and NPQ/4 values on the Area of Interest |
| 18. | Laptop computer | - | To display and analyzes the data acquired by I-PAM |

### 3.3. Research method

This research was based on laboratory experiment method. Marzuki (1977) stated that an experimental research is a research that explains the reason of undergoing processes or the effects of a certain condition applied.

Data were collected by sampling. Based on Supratno (2000), sampling is a data-collecting method which the investigated samples are elements or part of

a population. Even if the data collected was an estimation value, compared with census method, sampling is more economical, efficient in time and effort, and results in a more detail and a wider range of data.

### 3.4. Sampling Methods

Samples are taken under a stratified random sampling. Marzuki (1977) stated that stratified random sampling is a method in which every elements of the population has the same chance to become a sample, by previously stratificate the heterogeneous population into sub-population.

Three coral samples were randomly taken from Heron island reef flat form each coral of *Acropora aspera* (branching coral) and *Favites abdita* (massive coral) which are expected representing population of both species. Sample length taken varies from four to five cm. Two samples of each species colony representation were taken for fluorescence and zooxanthellae cell measurements.

### 3.5. Sample Measurements Method

#### 3.5.1. Photosynthetic Parameters Assessments on Coral Samples

One of the research facilities in Heron Island Research Station is the Pulsed Amplitude Modulation (PAM) fluorometer system, using MAXI Imaging-PAM device (Heinz-Walz GmbH, Germany). The system allowed us to conduct a non-invasive analysis on chlorophyll-fluorescence that occurred in an apparatus that undergoes photosynthesis. This method allowed us to apply saturated lights that give temperature influences to photosynthetic processes of zooxanthellae cells inside the tissues of reef-building corals.

Before measured by MAXI I-PAM, colony samples were adapted in dark condition (Jones and Hoegh-Guldberg, 1998) for 30 minutes therefore Photosystem work will stop temporary. Light treatment were then given by increasing intensity of saturation illumination light pulses to coral samples varies between 0-1251 µmol quanta $m^{-2}s^{-1}$ with 780 nm invincible light wave (close to infra-red) (Heinz-Walz, 2005). The increasing PAR intensity was given every 20 second starting from 0 µmol quanta $m^{-2}s^{-1}$.

Fluorescence image records were illustrated by ImagingWin version 1.01 software. Fluorescence areas measured were taken from AOI circle (Areas of Interest) (Figure 15) based on branch tip and basal area (*A. aspera),* mouth and septa-costae (*F. abdita*). Y(II) and NPQ/4 variable for every light treatment can be shown both in graphics and tables.

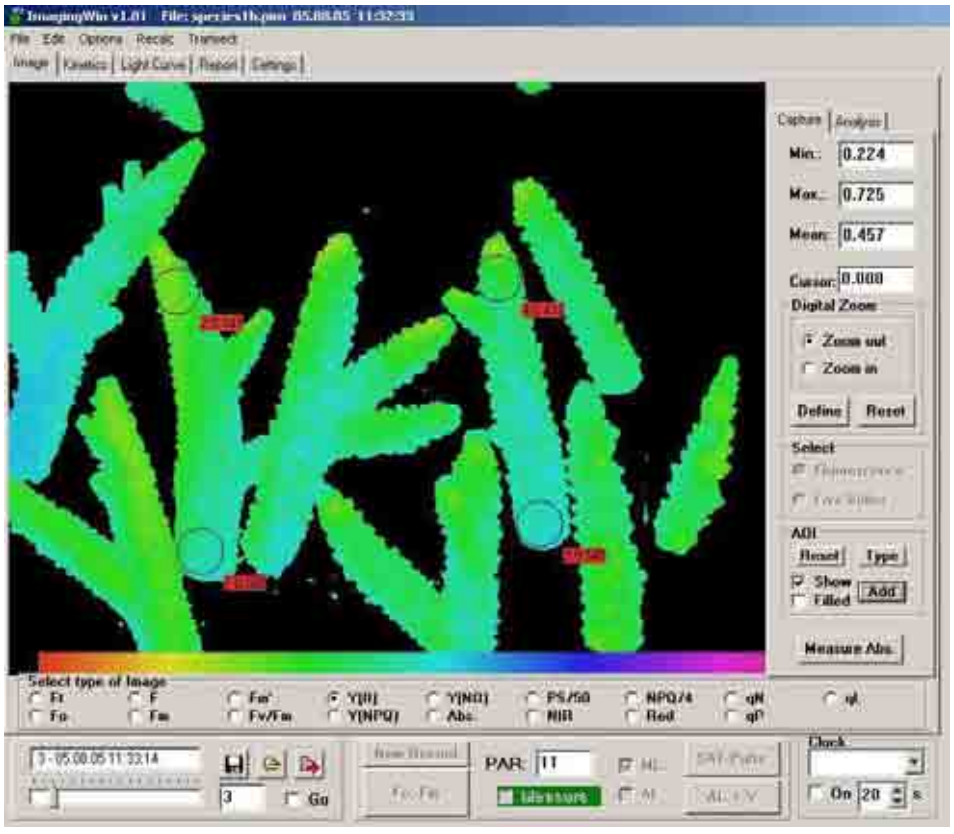


Figure 15. Fluorescence imaging display from coral samples

### 3.5.1.1. Effective PS II Quantum Yield (Y(II))

The values of effective PS II Quantum Yield are measured based on Genty *et al*. (1989) in Heinz Walz (2005) by the equation:

$$Y(II) = (Fm'-Ft)/Fm'$$

Note: Y (II) : Effective Quantum Yield value of samples that are saturated by light treatments

Fm' : Maximum Fluorescence Yield, a value of maximum fluorescence from each light treatment

Ft : Fluorescence Yield, a value of minimum fluorescence in each light treatment

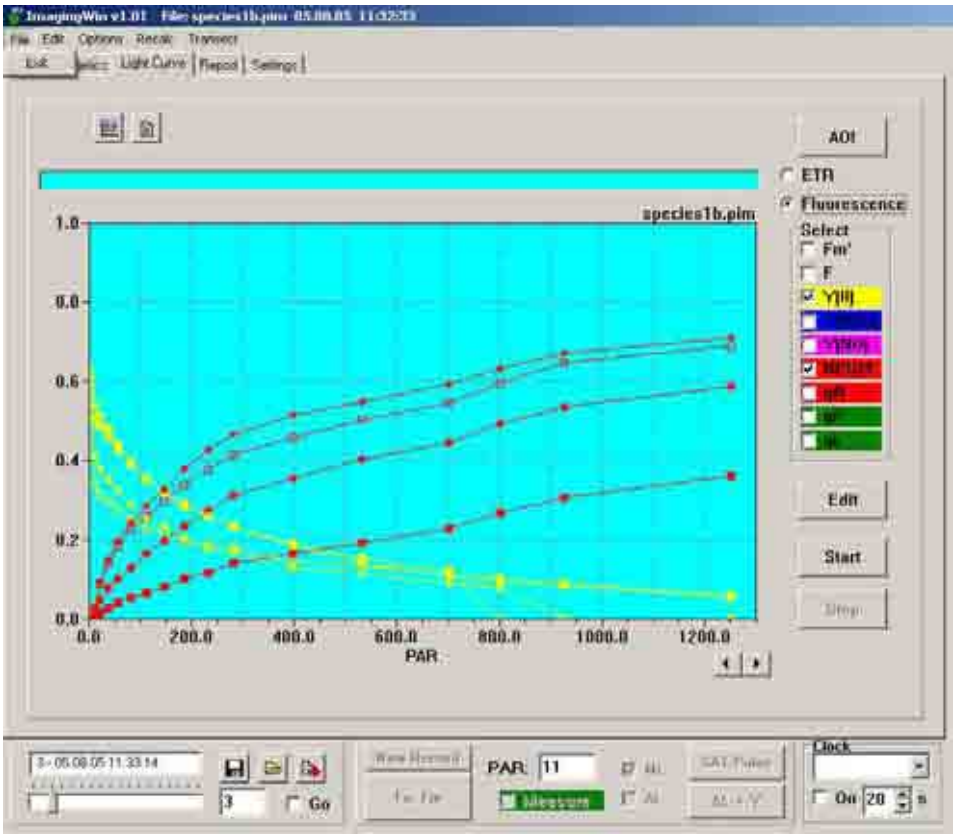


Figure 16. Graph display from values measured by I-PAM

### 3.5.1.2. Non-Photochemical Quenching (NPQ/4)

Values of Non-photochemical Quenching are count according to Heinz-Walz (2005), in the equation:

$$NPQ/4 = (Fm-Fm')/Fm'$$

Note: NPQ/4 : Non-photochemical Quenching value of the light saturated samples

Fm' : Maximum Fluorescence Yield, a value of maximum fluorescence in a light treatment

Fm : Fluorescence Yield, a value of maximum fluorescence in the first light treatment during dark-phase prior entering light-phase.

### 3.5.2 Zooxanthellae Cells Measurement Methods

#### 3.5.2.1. Cell Density

During the zooxanthellae cell measurements per unit sample area, at first, tissues of coral sample was released from its skeleton using a WaterPick™ (an airbrush connected to 50 ml reservoir of filtered seawater). Zooxanthellae cell densities were then measured in haemocytometer with 8 times replication. Normality of cell density was in n/ml. This measurement was based on a research method by Anthony and Hoegh-Guldberg (2003) in the equation:

$$\text{Zooxanthellae density (cell/ml)} = \frac{\left(\frac{n}{0.1}\right)}{5mm^2} x10^3$$

#### 3.5.2.2. Cell Size

Cellular morphology observation before light treatments in cell size were measured using binocular microscope equipped with 10 line scale graticule

eyepiece, and 100 x zoom in which would measures cell sizes in µm units. This measurement was based on the research method by Anthony and Hoegh-Guldberg (2003) in the equation:

$$\text{Cell size (µm)} = Mean(\sum_{1}^{20} nsize)$$

### 3.5.2.3. Chlorophyll Concentration per Cell

Absorbencies of isolated tissue resuspension during chlorophyll *a* measurements were using spectrophotometer in 630 and 663 nm wavelength,. The equation was based on Jeffrey and Humphrey (1975):

$$\text{Chlorophyll } a \text{ Concentration} = \frac{\left[\left(1.43xX_{630}\right)-\left(0.64xX_{663}\right)\right]x0.8x10^{21}}{CellDensity}$$

## 3.6 Data Analysis

Values of Y(II) and NPQ/4 which were obtained from photosynthetic parameters observation and zooxanthellae measurements of cell density, cell sizes and chlorophyll concentration per cell on *Acropora aspera* and *Favites abdita* were displayed and analyzed in forms of tables and graphics.

# CHAPTER IV

# RESULT AND DISCUSSION

## 4.1. Result

### 4.1.1. Y(II) Value Attainment

The Effective Photosystem II Quantum Yield (Y(II)) and Non-photochemical Quenching (NPQ/4) values obtained from *A. aspera* were measured by the mean values from AOI-s (Areas of Interest) from branch tip and basal areas, while on *F. abdita* measured from septa-costae / corallite wall and corallite mouth areas; executed in each coral colony sample (Mean and standard deviation of 2 replication). Zooxanthellae density was measured from eight repetitions, cell size were from 20 repetitions, and chlorophyll concentrations measured using 630 and 663 µm wavelengths.

#### 4.1.1.1. Colonies of *A. aspera*.

Fluctuations were observed on photosynthetic parameters of Y(II) and NPQ/4 values measured from three colonies of *A. aspera.* As the values of Y(II) tends to decrease, increments on NPQ/4 values were also occurred (Figure 17.a, 17.c, 17.e; based from data in Appendix 2 and 3) during increasing PAR treatment. Colony 1 Y(II) values has initially reached zero (at PAR=81 µmol quanta $m^{-2}s^{-1}$), followed by colony 2 (at PAR=1251 µmol quanta $m^{-2}s^{-1}$), while at PAR=1251 µmol quanta $m^{-2}s^{-1}$ colony 3 Y(II) value had not reached zero yet. The highest NPQ/4 values achieved, at PAR=1251 µmol quanta $m^{-2}s^{-1}$, were observed on colony 2 (0,632), following less (0,606), and 3 (0,304) afterwards.

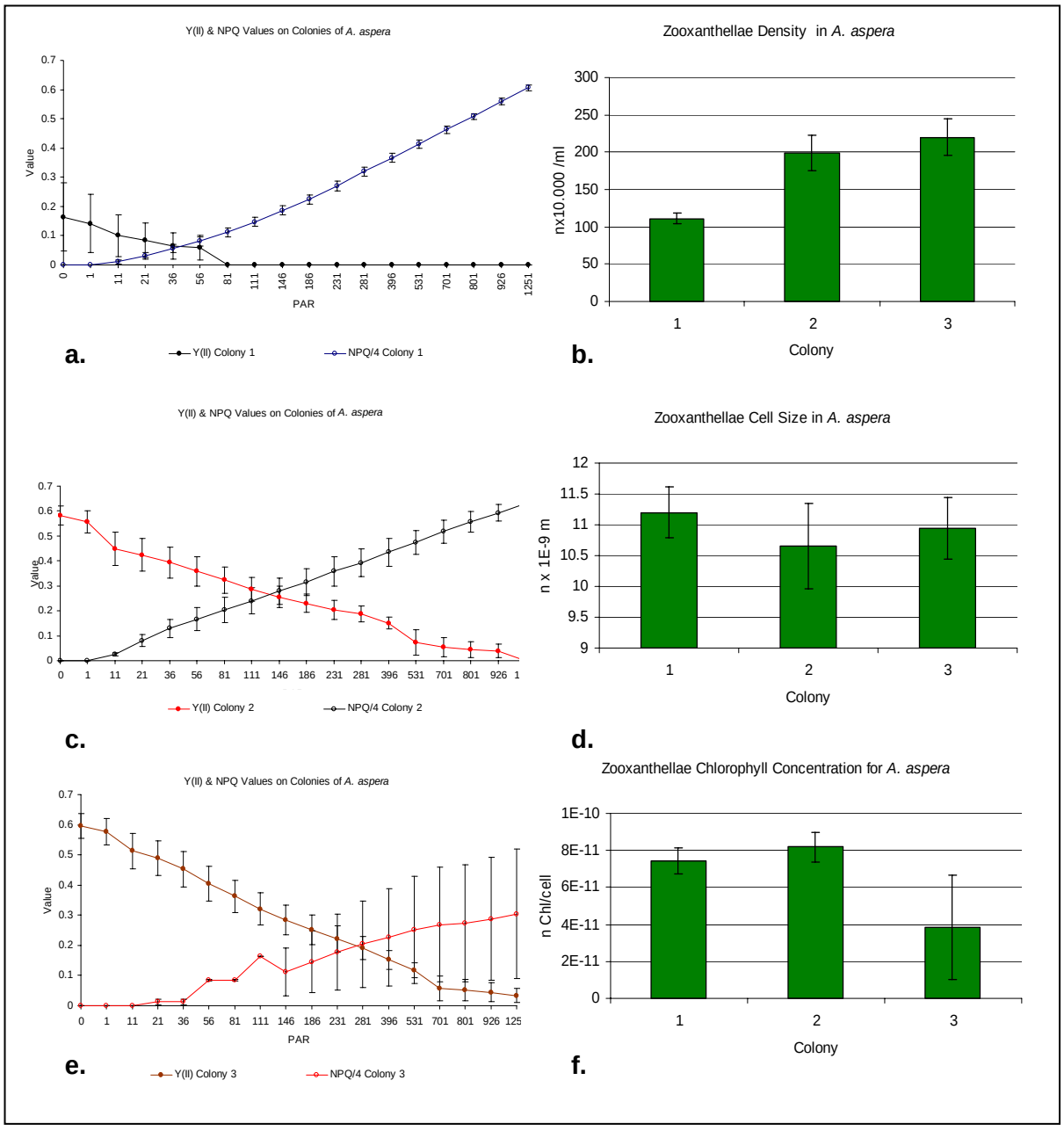


Figure 17. Data recordings of *A. aspera* retrieved from Maxi-I-PAM (graph) and Zooxanthellae measurements (histogram). Different result of Y(II) and NPQ/4 in colony 1, 2, and 3 (a, c, e), cell density (b), cell size (d), and chlorophyll *a* concentration (f).

Values of zooxanthellae cell density measured from *A. aspera* (Figure 17.b), highest to the lowest, were colony 3 (219,625 x $10^4$ cell/mL), colony 2 (119,125 x $10^4$ cell/mL), following last colony 1 (111,25 x $10^4$ cell/mL). Zooxanthellae cell size measured (Figure 17.d), highest to lowest, were on colony 1 (11,2 µm), colony 3 (10,95 µm), following last in colony 2 (10,65 µm). Chlorophyll *a* concentration in Zooxanthellae cell measured (Figure 17.f), highest to lowest, were on colony 2 (8,196 x $10^{-8}$ – chl/cell), colony 1 (7,448 x $10^{-8}$ chl/cell), and colony 3 (3,857 x $10^{-8}$ chl/cell) (see Appendix 6, 7 and 8).

#### 4.1.1.2. Colonies of *F. abdita*

Fluctuations of Y(II) and NPQ/4 values were also observed from three colonies of *F. abdita*. As values of Y(II) tends to decrease, increments on NPQ/4 values occurred (Figure 18.a, 18.c, 18.e; see data in Appendix 2 and 3) during increasing PAR treatment. However, an exception in colony 3 which was observed a reverse fluctuation of increasing Y(II) values, concurrently with decrement of NPQ/4 values, initially after 461 µmol quanta $m^{-2}s^{-1}$ PAR treatment.

Y(II) values attained zero was initially observed in colony 3 (at PAR=81 µmol quanta $m^{-2}s^{-1}$), following colony 2 (at PAR=701 µmol quanta $m^{-2}s^{-1}$), and last in colony 1 (at PAR=1251 µmol quanta $m^{-2}s^{-1}$). However, if mid-treatment of PAR=461 µmol quanta $m^{-2}s^{-1}$ was assumed as a constraint, then the highest Y(II) attainment would be in colony 2, following colony 1, and the lowest in colony 3. At PAR=1251 µmol quanta $m^{-2}s^{-1}$, the highest NPQ/4 value achieved were in colony 3 (0,841), following less in colony 2 (0,812), and last in colony 1 (0,663), whereas in the constraint of mid-treatment, similar colony stratification were also observed.

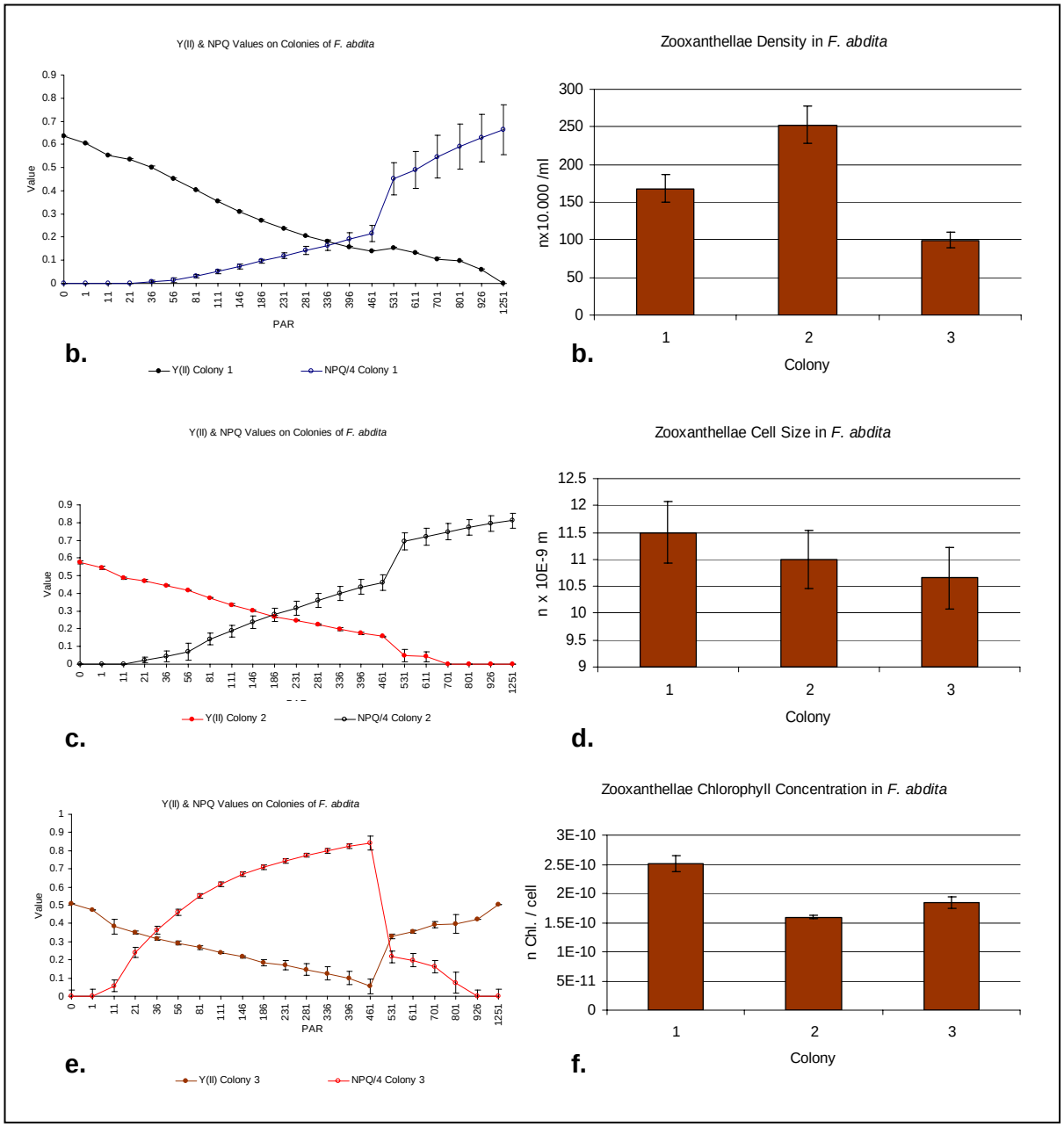


Figure 18. Data recordings of *F. abdita* retrieved from Maxi-I-PAM (graph) and zooxanthellae measurements (histogram). Different result of Y(II) and NPQ/4 in colony 1, 2, and 3 (a, c, e), cell density (b), cell size (d), and chlorophyll *a* concentration (f).

Table 2. Y(II) value attainment and zooxanthellae cell density (cell/mL) at PAR (µmol quanta $m^{-2}s^{-1}$) treatment constraints observed in colonies of *A. aspera* and *F. abdita* species.

| Species | Sample | Y(II) | Density | PAR Treatment |
|---|---|---|---|---|
| ***A. aspera*** | Colony 3 | 0,033 | 2.196.250 | 1251 |
| | Colony 2 | 0 | 1.991.250 | 1251 |
| | Colony 1 | 0 | 1.112.500 | 81 |
| ***F. abdita*** | Colony 2 | 0,156 | 2.522.500 | 461 )* |
| | Colony 1 | 0,136 | 1.680.000 | 461 )* |
| | Colony 3 | 0,054 | 992.500 | 461 )* |

Note : *) PAR Limitation by means of reverse fluctuations of Y(II)-NPQ/4 value.

Table 3. Y(II) values at the initial light treatment (PAR=0 µmol quanta $m^{-2}s^{-1}$) and final (PAR=1251 µmol quanta $m^{-2}s^{-1}$) from colony and inter-species observations on *A. aspera* and *F. abdita.*

| Species | Observation | Y(II) $_{PAR=0}$ | Y (II) $_{PAR=1251}$ |
|---|---|---|---|
| ***A. aspera*** | Colony 1 | 0.164 | 0.000 |
| | Colony 2 | 0.583 | 0.000 |
| | Colony 3 | 0.597 | 0.033 |
| | Species | 0.448 | 0.011 |
| ***F. abdita*** | Colony 1 | 0.635 | 0.000 |
| | Colony 2 | 0.574 | 0.000 |
| | Colony 3 | 0.510 | 0.506 |
| | Species | 0.573 | 0.169 |

Table 4. Y(II) values, chlorophyll concentration (n/cell), cell size (µm) and cell density at PAR treatments ranging 0 to 461 µmol quanta $m^{-2}s^{-1}$ observed on *A. aspera* and *F. abdita.*

| **Parameter** | ***A. aspera*** | ***F. abdita*** |
|---|---|---|
| $Y(II)_{PAR=0}$ | 0,101 | 0,143 |
| $Y(II)_{PAR=461}$ | 0,011 | 0,165 |
| Chl. concentration | $6,501 \times 10^{-8}$ | $19,82 \times 10^{-8}$ |
| Cell Size | 10,93 | 11,05 |
| Cell Density | 1.766.667 | 1.731.667 |

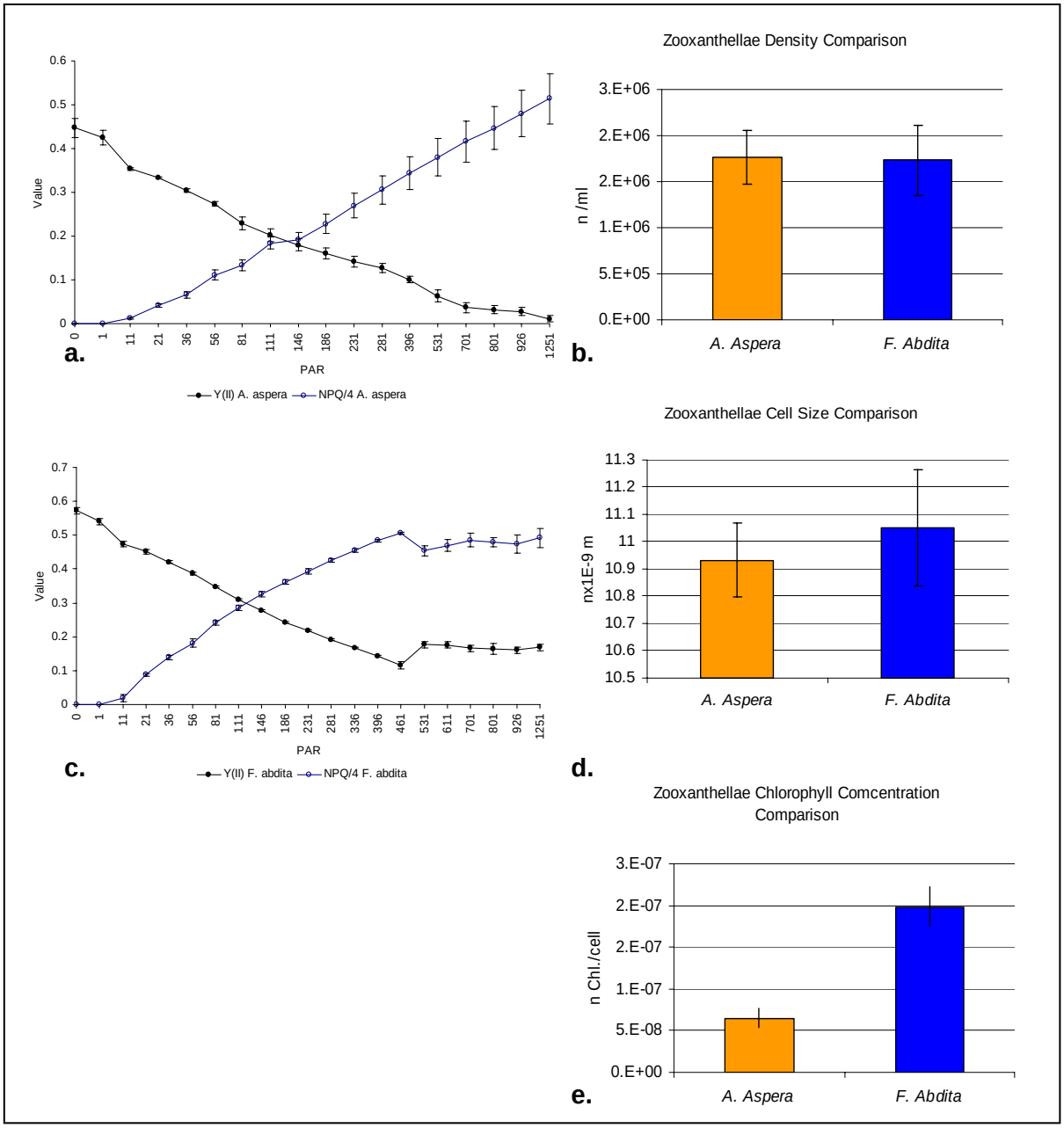


Figure 19. Result comparison between *A. aspera* and *F. abdita* retrieved from MAXI I-PAM (graph) and zooxanthellae cell measurements (histogram). Results of Y(II) and NPQ/4 values were based on mean values from colony 1, 2, and 3 (see data on Appendix 2, 3,4, and 5) on *A. aspera* (a), *F. abdita* (c), cell density (b), cell size (d), and chlorophyll concentration (e).

Table 5. NPQ/4 value achievement at 1251 µmol quanta $m^{-2}s^{-1}$ PAR treatment (*A. aspera*) and 461 µmol quanta $m^{-2}s^{-1}$ (*F. abdita*) in colony and species observation.

| Species | Observation | NPQ/4 |
|---|---|---|
| *A. aspera* | Colony 2 | 0,634 )* |
| | Colony 1 | 0,606 |
| | Colony 3 | 0,304 |
| | Species | 0,343 |
| *F.abdita* | Colony 3 | 0,841 )* |
| | Colony 2 | 0,463 |
| | Colony 1 | 0,214 |
| | Species | 0,483 |

Note : *) Highest value achieved form three colonies.

Zooxanthellae cell density measurement values on *F. abdita* samples (Figure 18.b), highest to lowest, were on colony 2 (252,25 x $10^4$ cell/mL), colony 1 (168 x $10^4$ cell/mL), following last colony 3 (99,25 x $10^4$ cell/mL). As for the zooxanthellae cell size (Figure 18.d), highest to lowest, were on colony 1 (11,5 µm), colony 2 (11 µm), following last colony 3 (10,65 µm). Chlorophyll concentration inside zooxanthellae cell (Figure 18.f), highest to lowest, were on colony 1 (2,510x$10^{-7}$ chl./cell), colony 3 (1,843x$10^{-7}$ chl./cell), and colony 2 (1,591x$10^{-7}$ chl./cell) (see data in Appendix 6, 7 and 8).

### 4.1.2. Inter-species Comparison

Values of Y(II), NPQ/4, cell density, cell size, and chlorophyll concentration of *A. aspera* and *F. abdita* were retrieved from mean values of three sample colony of each coral species (Mean and SD, n=3)

Y(II) values comparison on both species at the initial treatment (PAR=0 µmol quanta $m^{-2}s^{-1}$) showed that values on *A. aspera* (0,488) was lower that *F. abdita* (0,573). While at the mid-treatment constraint (at PAR=396 µmol quanta $m^{-2}s^{-1}$), where a sudden value fluctuation of Y(II) increment and NPQ/4 decrement, Y(II) values of *A. aspera* (0,100) was lower than *F. abdita* ( 0,143); where as the highest NPQ/4 value achieved in *A. aspera* (0,343) was also lower than *F. abdita* (0,506) (Figure 19.a, 19.c).

Zooxanthellae cell measurement result compared from both species also shown that the cell size (Figure 19.d) and chlorophyll concentration (Figure 19.e) from *A. aspera* (Cell size=10,93 µm, Chlorophyll concentration=$6,501x10^{-8}$/cell) was lower than *F. abdita* (Cell size=11,05 µm, Chlorophyll concentration=$19,82x10^{-8}$). While on the cell density result had shown a slight difference between *A. aspera (*1.766.667cell/mL ), which is higher, compared with *F. abdita* (1.731.667cell/mL) (see Table 4).

## 4.2. Discussion

The Effective Photosystem Quantum Yield values on this experiment was a variable/maximum fluorescence ratio, based on a research by Jones *et al.* (1998), which explains the photochemistry rate in the photosystem II (PS II) reactions, whereas in this research was used as a photosynthetic parameter on coral zooxanthellae.

Results between three colonies of *A. aspera* and *F. abdita* had shown a tendency that Y(II) values decreases concurrently as the zooxanthellae cell density stratification on colonies decreased (Table 2). This condition was assumed for the reason that the number of zooxanthellae contained inside may affect the photosynthetic efficiency on most corals, in branching coral as well as massive coral, (Levy et *al.* (2003) on *Stylophora pistillata, Favia favus, Plerogyra sinuosa, Goniopora lobata*) which explains that this relation has been initially observed on colony observation.

Levy *et al*. (2003) compared a colony of *Plerogyra sinuosa* (cell density= 2,744±1,16 $x10^6$ cell/mL) with *Favia favus* (cell density=0,198±0,083 $x10^6$ cell/mL). The result of a 30 µmol quanta $m^{-2}s^{-1}$ treatment (15 minutes) in light absorption (400-700 nm) for *P. sinuosa* (0-0.6) was higher than *F. favus* (0-0,2). And so did the range value of *P. sinuosa* in 460-660 nm wavelength were than *F. favus.* It was assumed that the high number of zooxanthellae cell in tentacle and mantle optimized the light absorbance efficiency that will be converted into energy on photochemistry process on photosynthesis.

Jones and Hoegh-Guldberg (2001) also observed that some colonies of *Stylophora pistillata* gives higher Y(II) value (0.75-1) with higher cell densities (5,804 - 8,944 $x10^6$ cell/ml) compared to other colonies (Y(II) = 0.5-0.75) with lower cell densities (3,162 - 5,804 $x10^6$ cell/ml).

The graphic of Y(II) value results from PAR treatments on colonies of *A. aspera* (Figure 17.a, 17.b, 17.c) and *F. abdita* (Figure 18.a, 18.c, 18.e) had shown a tendency of curve decline of Y(II) value towards zero value. A comparable condition was also observer in research by Jones *et al.* (1998) *in* (Hoegh-Guldberg, 1999) which explains that the increase of light (PAR)

intensity has become a stress effect to corals reaching the last treatment. A similar curve decline was also occurred, but on coral species of *Stylophora pistillata*, conducted by Hoegh-Guldberg and Smith (1989) *in* (Hoegh-Guldberg, 1999) that also explains the occurrence of photo-inhibition mechanism inside the algae but the lost of algae symbiont has not occurred yet (no bleaching).

Decrement of Y(II) values during increasing light treatment was also explained an early indication towards coral bleaching where the high light intensity treatments has caused a reaction function decline inside PS II (Iglesias-Pierto, (1995); Warner *et al*. (1996); Jones *et al.* (1996) *in* Hill *et al.* (2004). Both on colony observation and also inter-species during light treatments, from zero to last, the Y(II) value tend to decline towards zero value. A research by Levy *et al.* (2003) showed that as the light intensity increases the efficiency in the harvest of light quanta are likely reaching steady state so it would result in the increase of heat produced. Unprocessed electron by the reaction centre would also cause the formation of Reactive Oxygen Species (ROS); and finally the PS II reaction centre will be shut (Y(II)=0) (Hill *et al.,* 2004).

Different Y(II) values of each coral species has shown some photosynthesis heterogeneity which are commonly found in several coral studies (Kühl *et al.,* 1995; Ralph *et al.,* 2002; Hill *et al.,* 2004 *in* Ralph, *et al.,* 2005). Micro-climate variations of lights on coral colonies are also influenced by different skeletal structures (areas of polyps and coenosarc tissues on three species, Ralph et al., 2005) and variation of tissue thickness (Fitt and Cook, 2001) were also assumed to result different Y(II) between colonies.

Microclimate light has caused light dispersion as a result light reduction reaching chlorophyll *a* pigments.

Results from inter-species observations on *A. aspera* and *F. abdita* had shown different results compared with species colonies observations. Whereas the tendency of the Y(II) attainment value of *A. aspera* at PAR range of 0 to 461 µmol quanta $m^{-2}s^{-1}$, was lower than *F. abdita,* and was assumed to be parallel with the value of zooxanthellae cell size and chlorophyll *a* concentration of both species. While zooxanthellae cell density value of *A. aspera* was almost equal with *F. abdita* (see Table 4).

Results described before had shown that even though the zooxanthellae cell density value of *F. abdita* was lower than *A. aspera*, the high chlorophyll *a* per cell concentration of *F. abdita* has able to result a higher photosynthetic capacity which was indicated with the higher Y(II) value attainment of *F. abdita.*

Chlorophyll, a green pigment inside the chloroplast, has a function to utilize the lights absorbed for synthesizing food molecules through photosynthesis (Campbell, 1990). Based on Lakitan (2001), the PS II reaction centre is activated by the chlorophyll *a* type P680 pigment that acts as an antenna and harvests the light with wavelengths reaching 680 nm also as an acceptor of energies absorbed from other pigments. For that reason, based on Schreiber (1997), it was assumed that more pigment means more antennas to convert the light into energy, by photosynthesis, would also increased, which is indicated with a high fluorescence yield.

Inside the body of a zooxanthellae cell, similar as a common plant cell, holds chloroplast organelle that contains chlorophyll pigments (Campbell,

1990). Observed between zooxanthellae from both coral species, a bigger cell size of *F. abdita*, compared with *A. aspera,* was assumed as a result of higher amount of chloroplast organelle inside the cell. This condition was expected also as a reason of a higher chlorophyll *a* concentration on *F. abdita*.

As an implication, this discussion has assumed that *A. aspera* is more vulnerable against high light intensity stress which would result a higher susceptibility on coral bleaching, rather than *F. abdita.* As it observed by Hill *et al.* (2004), that a low Y(II) value attainment would result an earlier damage in the PS II component, which would apply on coral samples *A. aspera* during increasing PAR treatments.

Values achieved on NPQ/4 from results in species colonies, was the highest from colony 3 of *F. abdita* for 0,841 while the highest achieved from *A. aspera* in colony 2 which was lower for 0.634. As well as the result from inter-species comparison, NPQ/4 value achieved by *F. abdita* was for 0,843 while *A. aspera* was only 0,343 (see Table 5). As stated by Hill *et al*. (2004), that NPQ values would explains the capacity of PS II reaction centre in dissipating the heat as a result of high light intensity stress. Therefore, it is assumed that F. abdita has a better heat dissipation regulation than A. aspera.

Results showed an increase of NPQ/4 values together with the decreasing Y(II) value during light treatments, similar result also shown in former research by Warner *et al.* (1996) *in* Hill *et al.* (2004). The research (on *Acropora nobilis*) also shown that non-photochemical quenching was proportional with the light increase treated on corals, while the PS II reaction centre was also declined (decreasing PS II yield). Basically, the quenching mechanism would initially occurred to dissipate the heat, and PS(II) process

would decelerate in light harvesting down to reduce heat production (Lesser and Gorbunov, 2001)

Research from Hoegh-Guldberg and Jones (2001) has also showed that inside the coral algae symbiont (*Symbiodinium spp.*) contains chlorophyll *a* and *c* as a light capturing pigment together with the xanthophylls component that also dissipates heat from PS I to PS II to xanthophylls pigments (Lesser and Goburnov, 2001). The light capturing pigments in the photosystem would gradually change as a result of acclimatization for light intensities increment (Hoegh-Guldberg, 1999). Therefore, it is probable that the low NPQ/4 value achieved on *A. aspera,* compared with *F. abdita,* for the reason that *A. aspera* also has a lower chlorophyll *a* concentration that cause a low capacity in heat dissipation.

Heat energy dissipation inside PS II, during photochemical process in reaction centre, was occurred by diverting unprocessed excess light quanta/photon to xanthophylls to carry out quenching cycle. The cycle would go be de-excitation photons that have been diverted to xanthophyll pigments (Demmig-Adams (1990) *in* Jones *et al*. (1998)).

Based on the research of Havaux et al. (1996); Tardy and Havaux (1997); Havaux and Niyogi (1999) *in* Brown *et al.* (2002) on higher plants, it was also shown that the presence of xanthophylls has other benefits apart from NPQ. These include the stabilization and protection of chloroplast thylakoids during both heat and light stress. By this statement, it is assumed the high achievement of NPQ/4 value on *F. abdita,* compared with *F. abdita* was also caused by the high amount on xanthophylls pigments contained inside the residing zooxanthellae chloroplast.

Based on Brown *et al.* (1997), difference in NPQ/4 achievement between colonies in each coral species sample was assumed as a result of modification in xanthophyll pigment density which runs the quenching mechanism as an acclimatization of high light intensity.

Reverse fluctuation of Y(II) and NPQ/4 values occurred during PAR treatment in colony 3 of *F.abdita* which had only shown a rise in Y(II) and NPQ/4 down to zero only from 461 to 1251 µmol quanta $m^{-2}s^{-1}$. This condition was assumed also because of coral acclimatization against light, some of the probabilities are: 1) Increasing number of chlorophyll (Falkowsky and Dubinsky, 1981 *in* Hoegh-Guldberg, 1999) and xanthophyll pigment (Brown *et al.,* 1999), 2) Coral polyp tissue retraction and 3) Biochemical defence (anti-oxidative enzymes) (Brown *et al.,* 2002).

Hoegh-Guldberg (personal correspondence) sated that during initial six minutes of PAR treatments, in this research, was possible for xanthophyll number to increase. Increasing number of xanthophyll pigment may allow more efficient quenching (NPQ/4 value decrease) of excessive heat from PS II reaction centre, so that the photochemical process in PS II can regulates again (Y(II) rise).

Former discussion has resulted an assumption that zooxanthellae within *F. abdita* has a better heat dissipating ability in PS II component, compared with zooxanthellae within *A. aspera.* Based with the indications of stress towards bleaching from NPQ process attainment during increasing light treatment (Jones *et al.,* 1998; Brown *et al.,* 2002; and Hill *et al.,* 2004), it is assumed that *A. aspera* would have a higher susceptibility to coral bleaching than *F. abdita,* in the causal of high light intensity stress. It was claimed also that those corals more capable of

dissipating excess excitation energy through non-photochemical quenching are less prone to temperature-induced bleaching (Warner et al., 1996).

# CHAPTER V

# CONCLUSIONS AND SUGGESTIONS

## 5.1. Conclusions

Conclusions of this research on photosynthetic parameter assessments on coral zooxanthellae in coral species of *Acropora aspera* and *Favites abdita* are*:*

1. Throughout PAR treatments ranging from 0 to 1251 µmol quanta $m^{-2}$ $s^{-1}$ applied on three colonies of *A. aspera* and *F. abdita* had shown a fluctuation tendency of Y(II) value decrement in parallel with NPQ/4 increment.
2. Results from three colonies if *A. aspera* has assumed that Y(II) value attainment was related in parallel zooxanthellae density number based from the colony stratification values, highest to lowest, of colony 3, colony 2, and colony 1.
3. Results from three colonies if *F. abdita* has also assumed that Y(II) value attainment was related in parallel with zooxanthellae density number based from the colony stratification values, highest to lowest, of colony 2, colony 1, and colony 3.
4. Result from inter-species comparison value of *A. aspera* and *F. abdita* has also assumed that Y(II) value attainment and NPQ/4 value achievement were correlated in parallel with the value of zooxanthellae cell size and chlorophyll concentration.
5. Based from inter-species comparison value of *A. aspera* and *F. abdita* has also shown that *A. aspera* has a lower Y(II) value attainment and

NPQ/4 value achievement compared to *F. abdita,* during PAR treatments ranging from 0 to 1251 mol quanta $m^{-2}$ $s^{-1}$.

## 5.2. Suggestions

As a follow-up, a similar research may be conducted focusing in on tropical water corals. A further study is also needed to find out types of zooxanthellae that reside within coral species of *A. aspera* and *F. abdita.* We hope all of these efforts may possibly be useful in taking part of the global coral reef management, particularly in the study of coral resistance and resilience facing the increasing global warming stresses ahead.

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