The effect of light and nitrogen on the lipid and carotenoid production in *Symbiodinium*

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Abstract. The marine photoautotrophic and endosymbiotic organism, *Symbiodinium*, uses a combination of solar radiation and nutrient as its principal sources to maintain its physiological processes which include photosynthesis, growth, and intracellular chemical compound production such as lipid and carotenoid. The present study examined the synergetic effect of light and nitrogen on the intracellular lipid production and carotenoid accumulation in *Symbiodinium*. The pure and isolated *Symbiodinium* was cultured and treated with a combination of light intensity and nitrogen depletion. Data such as biomass growth, lipid content, and carotenoid in its initial culture condition were analysed to examine their correlation. The results showed that the initial stresses reduced biomass, increased total lipid content which was dominated by palmitic acid (C16:0), linoleic acid (C18:2n6), and linolenic acid (C18:3n3), and increased the intracellular carotenoid of *Symbiodinium*. The research identified a highly positive linear correlation between intracellular lipid production and carotenoid accumulation of *Symbiodinium* in initial culture condition.

Key Words: environmental effect, lipid, photopigment, *Symbiodinium*.

Introduction. Since early nineteenth century, global alternative energy resources have become a crucial factor in the industrial revolution in intensifying the economic growth and living standard (Atabani et al 2012). Human beings are making the effort of balancing the economic and ecological needs by searching for efficient and abundant biosources to provide for a better future to live in. Marine microalgae are potential biosources of biofuel and other fine biochemical compounds due to the higher growth rate and high lipid content of their cellular structure (Prartono et al 2013; Hudaidah et al 2015). The variability of microalgae species and its culture conditions are the two most important variables that influence oil production (Wang et al 2008; Xu et al 2013). It has been reported that marine microalgae are the more sustainable source of biodiesel in terms of food security and environmental impact (Prartono et al 2013; Wang et al 2013; Hudaidah et al 2015).

Nitrogen is a vital environment element which quantitatively influences highly the variability of microalgae growth and lipid productivity within the diverse free living marine microalgae (Griffiths & Harrison 2009; Prartono et al 2010). Furthermore, the modified light intensities may play an important role in increasing the lipid content of microalgae (Rosenberg et al 2008). Initially, the lipid production of the most common cultured microalgae ranges between 20 and 50% and is affected by the nutrient variability; subsequently, light intensity has been identified as another potential factor to regenerate their high lipid productivities (Mata et al 2010).

The marine microalgae found in both free living or endosymbiotic microalgae may potentially serve as biofuel and sound chemical compound sources. The marine free-living microalgae *Nannochloropsis* sp. are considered to be suitable for biofuel production because of their high oil content (26.2-28.7%, respectively) (Gouveia & Oliveira 2009; Hudaidah et al 2015). The average lipid production of marine microalgae may vary

during their initial culture conditions; certain species may even consist of up to 90% dry weight of lipid content (Chen et al 2015). Prartono et al (2013) showed the potential use of other free-living unicellular marine microalgae (*Skeletonema, Thalassiosira,* and *Chaetoceros*) as biodiesel sources by analysing their lipid content and composition under controlled and stressed conditions. The dynamic model analysis of microalgae described the high correlation of light and nutrient (as independent factors) alongside their growth and intracellular chemical compound production (Tang et al 2000). On the other hand, light may affect the physiological behaviours of those corals which contain endosymbiotic microalgae (*Symbiodinium*). Moya et al (2006) showed that light has simplified correlation with the calcification process in their host coral. Furthermore, appropriate lighting condition is essential for the production growth of sound chemical compound and maintenance of endosymbiotic microalgae biomass of gastro dermal host corals (Krueger & Gates 2012).

Symbiodinium may be found as marine free-living and endosymbiont microalgae (Baker 2003; Kuguru et al 2010). *Symbiodinium*, as the most abundant marine Cnidarians endosymbiont, a unique phenomenon can be observed in its interaction between the gastro dermal cells of host and their photoautotrophic symbiotic microalgae. This mutual relationship plays a critical role in maintaining the health condition of the host and lays the foundation of the tropical marine ecosystem (Finney et al 2010; Peng et al 2012). *Symbiodinium* could be a potential biofuel source due to its significant ability to produced intracellular fine chemical compounds (Krueger & Gates 2012). Only a few studies have examined the content, synthesis, and biological activity of fatty acids in *Symbiodinium* (Wang et al 2013; Chen et al 2015; Liang et al 2015). As a marine microalgae, *Symbiodinium* may be influenced by marine environmental factor such as nutrient and light availability. Nutrient limitation and high light intensity, as stress variables, separately may induce lipid accumulation during cyst formation in *Symbiodinium* (Krueger & Gates 2012*;* Liang et al 2015). These conditions tend to fluctuate the photosynthesis process of pigments and change other physiological performances (Strychar & Sammarco 2012). Compared to terrestrial microalgae, a brief significant inverse correlation between intracellular lipid production and photosynthetic activity was briefly described in the freshwater microalgae *Chlorella* sp. (White et al 2011). Furthermore, Solovchenko et al (2010) stated that the tight, nonlinear relationship between the photo pigment: chlorophyll ratio and total fatty acid (TFA) contents per dry weight occurred during the cultivation of *Parietochloris incisa*.

Although the potential lipid/biofuel production in free-living micro algae have been investigated as mentioned above, there is still a lack of the studies in examining the lipid and photo pigment production strategy under specific conditions in endosymbiotic micro algae represented by *Symbiodinium*. In this research, we examined the lipid production and the relationship between carotenoid accumulation under nitrogen depletion and light intensities treatments in *Symbiodinium* natural biomass.

Material and Method

Period of study. The research was done in two major steps; the preparation and the main research. The preparation steps consist of host sample collection, and isolation of *Symbiodinium* which were conducted on January-July 2017. The main research consist of analysis of lipid extraction, Total Fatty Acid (TFA) analysis, and photo pigment analysis which were conducted on August-October 2017.

Collection of host and isolation of Symbiodinium. The symbiotic *Fungia* sp. was collected from Marine Protected Area (MPA) of Pahawang island, Lampung Province, Indonesia as sources of *Symbiodinium* samples. The specimens were brought into the laboratory within < 9 h after being collected. The targeted zooxanthellae were collected from ±1 cm piece of host tentacles and homogenized in 0.45 µm millipore filtered sea water (MFSW) by using Teflon and glass homogenizer. The crude suspension was washed twice by centrifugation (~500 rpm for ~2 minutes) and resuspended in MFSW (Perez et al

2001). A spectrophotometer approach was used to calculate the *Symbiodinium* biomass for all treatments according to Zamani & Muhaemin (2015).

Symbiodinium culture conditions. The endosymbiotic marine microalgae, *Symbiodinium,* was cultured in the Laboratory of Fisheries and Marine Science Research, University of Lampung. The acclimation was conducted at 21±1^{o}C and cultured in a semi outdoor running seawater tank and was fully exposed with an ambient natural solar radiation with a photo period ratio of 12:12 light:dark. The maximum photosynthetic photon flux density (PPFD) exposed to the specimens was 600-700 nm (~2100±50 µmol m^{-2} s^{-1}). The preparation and composition of culture media were based on Krueger & Gates's (2012) formula (KGF) which then were added with (NO_3^-/NH_4^-) as nitrogen source, chelating agents and organic supplements. The bio-contamination of diatom was reduced by omitting silica and tris-base compound from all culture media.

After the acclimation process, the surviving endosymbiont microalgae biomass with exponential growth phase was used for the experiments. The microalgae cells were harvested by centrifugation (4500 rpm for 4 min), washed twice with double-distilled water, and re-suspended for all preliminary treatments. The treatments conditions were (1) control (the KGF medium was given the same condition as indicated in the acclimation process); (2) the KGF medium was exposed under 450 µmol photons m^{-2} s^{-1} (N_2-HL); (3) the KGF medium was nitrogen-free and treated under optimal light intensity (without N_2); and (4) the KGF medium was nitrogen-free and treated under 450 µmol photons m^{-2} s^{-1} (without N_2-HL). Each experiment was performed for 12 days of culture (DOC) with at least three independent repetitions for each treatment were conducted. The *Symbiodinium* cells were sampled every 24 hours for the analyses.

Lipid extraction and total fatty acid (TFA) analysis. *Symbiodinium* cultured cells were harvested and lyophilized by using a freezer dryer to analyse the total lipid content. A total of 30 mg of freeze dried samples were mixed well with 1000 µL of methanol at 4°C for 20 minutes. A triplicate extraction was done to obtain all the neutral lipids. Subsequently, the methanol was extracted (containing 0.01% butylhydroxytoluene, hexane, and water) with a final ratio of 1:1:1 (v/v/v). There are two phases involved in this analysis. The collection of *upper phase* was conducted after mixture underwent centrifugation (2500xg for 10 min). The pH of the *lower phase* was adjusted to 3.5-4.5 with acetic acid, and re-extraction with a mixture of diethyl ether and hexane (1:1 v/v) was conducted. Subsequently, the combined phases evaporated to dryness in nitrogen and stored at -25°C. Four different treatments were performed after 12 days and three independent repetitions for each extraction were conducted to minimize response bias between treatments.

The fatty acid content was analysed by providing 20 mL of each lyophilized sample and placing these samples into a conical flask containing 30 mL of petroleum ether (An et al 2013). Each solution was placed in an ultrasound bath equipment (40.0 kHz and 600 W) for 35 min at 45°C. This process was repeated twice. Another option would be to move the solvent from a rotary vacuum evaporator at 45°C until the weight was stable. The total fatty acids content was transmethylated to fatty acid methyl esters (FAMEs) with 6 mL of 0.35 M KOH:methanol (v/v) at room temperature (18±1°C). The FAMEs treatment was carried out using a Finnigan Trace GC-MS (Agilent Technologies, USA). The FAMEs were analysed by comparing authentic standards (Sigma Chemicals Co., USA), against peaks that were simulated with DPS software Version 7.05.

Photo pigments analysis. Photo pigment was analysed by harvesting stress-exposed and ultrasonic decomposition of 50 mL of *Symbiodinium* culture at different intervals. The photo pigments were extracted from microalgae cells by adding dimethyl sulfoxide (DMSO) at 70°C for 5 min (Strychar & Sammarco 2012). The total carotenoid and chlorophyll contents were examined by UV-Vis spectrophotometers (Purkinje General, China) using the coefficients mentioned by Solovchenko et al (2010). The chlorophyll and carotenoid concentrations were expressed in mg of carotenoid per litre, respectively.

Statistical analysis. Each experiment was repeated three times. All observations and calculations were made separately for each set of experiment. The data were expressed as means (μ) combined with standard deviation (SD). Statistical analyses were performed by using SPSS 17.0 for Windows. Variance among treatments was tested using a one-way ANOVA. The significance level was $p < 0.05$ for all tests unless otherwise indicated.

Results. In the control culture, the sample showed a maximum cell concentration of 8.38×10^6 cells mL^{-1} after 10 DOC and reached an exponential phase from the 2nd to the 8th DOC. *Symbiodinium* cultured in [HL] treatment entered the exponential growth phase after 2 DOC with the highest cell concentration of 4.24×10^6 cells mL^{-1} after 10 DOC. Both [without N_2] and [without N_2-HL] cultures showed no exponential growth phase. The maximum cell concentrations in [without N_2] and [without N_2-HL] were 1.85×10^6 cells mL^{-1} after 11 DOC and 1.54×10^6 cells mL^{-1} after 10 DOC, respectively.

Figure 1 shows that all treatments exhibited various increase in biomass yield, even though the cultures were exposed to nitrogen and light stresses. All treatments showed lower cell concentrations than the control cultures. The cells under nitrogen depletion treatment and the nitrogen depletion combined with the high light treatment did not exhibit an obvious exponential phase (Figure 1). The culture stressed with nitrogen depletion had a significantly reduced biomass of *Symbiodinium* than the cultures handled by other treatments.

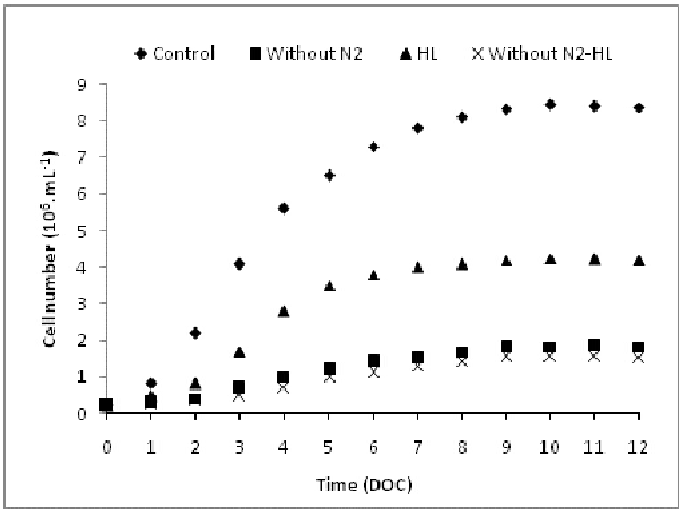

Figure 1. The growth of *Symbiodinium* in the control-, HL-, without N_2-, and without N_2-HL cultures within 12 DOC of cultures [HL refers to the cells under high light (450 μmol photons m^{-2} s^{-1} of continuous light); without N_2 refers to the cells under nitrogen starvation stress; without N_2-HL refers to the cells under high light (450 μmol photons m^{-2} s^{-1} of continuous light) and nitrogen starvation stresses].

Figure 2 showed the total lipid contents of *Symbiodinium* during the treatments. In the control condition the total lipid content of *Symbiodinium* gradually increased with the rise of DOC in linear model resulting in a maximum total lipid content of 13.6% dw at the 10th day. The total lipid content in [without N_2] and [without N_2-HL] cultures increased sharply in the first 6 DOC and raised slowly in the following 4 DOC, then declined after the 10 DOC. Thus, the highest lipid productivities of [without N_2] and [without N_2-HL] cultures were obtained after 10 DOC, allowing for a production of up to 45.2% and 46.3% respectively. In the [HL] culture, a sharp increase of total lipid content in the first 8 DOC was observed. In the following 2 DOC a similar increase was detected, although less pronounced. In the last 2 DOC, the total lipid content decreased slightly.

Figure 2 showed that the highest amount of total lipid content obtained after 10 DOC was significantly higher than in the longer cultivated culture. The maximum productivity for total lipid accumulation in high-light-exposed cells was 56.92%, which was almost six-fold higher than the control cells. The cellular contents of total lipid

declined after 10 DOC regardless of any culture condition. The results suggest that the 10th stress day may be the optimal time for lipid production.

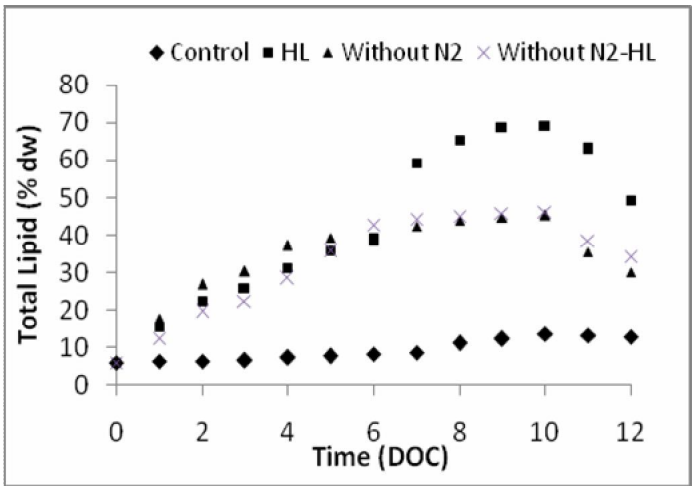

Figure 2. Total lipid content (% dry weight) in *Symbiodinium* grown within the control-, HL-, without N_2-, and without N_2-HL-treatments.

Table 1 showed the change in the fatty acid (TFA) composition of the *Symbiodinium* cells growth. The dominant fatty acids in *Symbiodinium* were palmitic acid (C16:0), linoleic acid (C18:2n6), and linolenic acid (C18:3n3), which were found to represent about 65-72% of the TFA. Generally, the TFA was increased under stress conditions except under [without N_2] treatment compared with control. Additionally, significant increase were found in oleic acid (C18:1) which rose by 3.1-fold under [HL], 2.2-fold under [without N_2], and 2.44 fold under [without N_2-HL] treatments culture condition. The different phenomena were obtained by the decreased of C16:2, C16:4, C18:3n6, ARA, and EPA fatty acids.

Table 1

Fatty acid species profile (%) in the control, [HL], [without N_2], and [without N_2-HL] treatments culture

Fatty acid species	*Treatments*			
	Control	*HL*	*Without N_2*	*Without N_2-HL*
C14:0[a]	0.45±0.04	0.67±0.02	0.40±0.02	0.55±0.04
C16:0[a]	24.2±0.09	26.59±0.01	26.36±0.10	27.62±0.11
C16:2[a]	2.15±0.08	0.49±0.02	0.39±0.03	0.00±0.00
C16:3[a]	3.38±0.05	1.14±0.03	0.70±0.03	0.38±0.02
C16:4[a]	9.89±0.04	7.44±0.03	8.05±0.03	7.82±0.01
C18:0[a]	3.29±0.01	3.43±0.04	2.47±0.02	3.06±0.01
C18:1[a]	3.14±0.03	9.60±0.05	6.88±0.07	7.57±0.02
C18:2n6[a]	17.06±0.03	17.22±0.04	18.11±0.06	19.27±0.08
C18:3n6[a]	1.30±0.04	1.10±0.03	1.12±0.02	0.87±0.04
C18:3n3[a]	19.49±0.04	19.70±0.04	20.38±0.02	19.94±0.01
C18:4[a]	3.70±0.04	3.72±0.05	3.06±0.06	3.69±0.06
C20:4n6 (ARA)[a]	2.79±0.05	1.56±0.05	1.40±0.5	1.42±0.04
C20:5n3 (EPA)[a]	1.91±0.03	1.41±0.08	1.28±0.01	1.44±0.01
ΣSFA[b]	27.89±0.05	30.69±0.07	29.23±0.10	30.23±0.06
ΣMUFA[b]	3.14±0.03	9.60±0.05	6.88±0.07	7.57±0.02
ΣPUFA[b]	61.67±0.44	53.78±0.26	54.49±0.35	54.83±0.60

[a]Values are means±SD of three determinations; [b] SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids. HL refers to the cells under high light (450 µmol photons m^{-2} s^{-1} of continuous light); without N_2 refers to the cells under nitrogen starvation stress; without N_2-HL refers to the cells under high light (450 µmol photons m^{-2} s^{-1} of continuous light) and nitrogen starvation stress.

The initial percentage of saturated fatty acids (SFA) was shown under [HL]- (10.04%), [without N_2]- (4.81%) and [without N_2-HL] culture (8.39%) conditions compared to the

control one. The monounsaturated fatty acid (MUFA) content increased higher than others such as [HL] culture (205.73%), [without N_2] culture (119.11%), and [without N_2-HL] culture (141.08%) respectively. The different phenomena shown by the poly-unsaturated fatty acid (PUFA) content presented a downward trend.

The dynamic changes in carotenoid content are presented in Figure 3. The effect of the synergetic stress of nutrient and light on carotenoid concentration was examined to prove the linkage between the accumulation of lipid and carotenoid. The intracellular content of the total carotenoid in the control culture tends to stabilize appreciably within 12 DOC. In [without N_2] culture, the total carotenoid content increased moderately. In [HL] culture, the content in total carotenoid increased slowly on the first 6 DOC, continues to rise exponentially between 6 and 10 DOC, and beyond that will start to decrease (Figure 3).

Generally, the intracellular carotenoid concentration peaked on the 10th day, to 29.90 mg L^{-1} in [HL] cultures and to 22.10 mg L^{-1} in [without N_2] cultures, respectively. The intracellular carotenoid concentration in [without N_2-HL] culture was nearly three times higher than the one in the control culture (Figure 3).

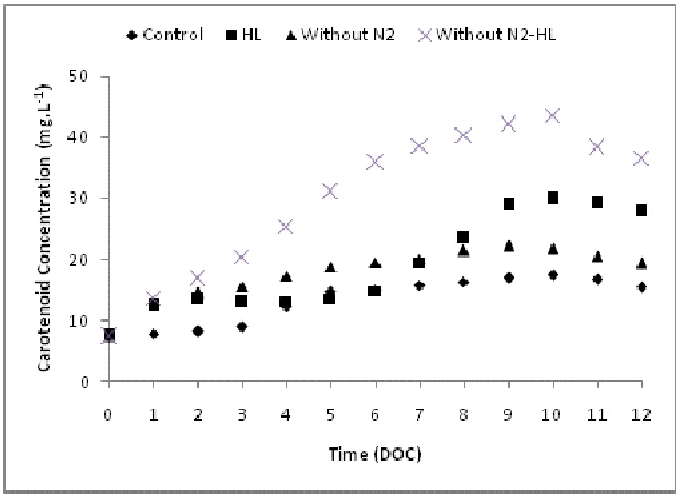

Figure 3. The fluctuation of total carotenoid contents of *Symbiodinium* cells growth in the control-, [HL]-, [without N_2]-, and [without N_2-HL] cultures.

Discussion. In this research, an assumption was made to compare the changes in lipid content and carotenoid profile in *Symbiodinium* under various stress conditions. The trend of biomass accumulation observed in *Symbiodinium* (Figure 1) was comparable with those from previous observations (Damiani et al 2010; Fitriyani et al 2017). The intracellular concentration of fatty acids under nitrogen starvation is a widely known phenomenon as mentioned in Ahmad et al (2011) and Fitriyani et al (2017). However, we were particularly interested in analysing the correlation between lipid accumulation and high light intensity in *Symbiodinium* cultures with or without nitrogen, and also whether stress conditions induced a sharp increase in the intracellular lipid content of *Symbiodinium*. The observations of biomass and lipid accumulation (Figures 1 and 2) were briefly described as mentioned in Gustavs et al (2010) that when microalgae were cultured under stress condition, the occurrence of the intracellular degradation are correlated with the fluctuation of intracellular cellular C/N balance, which apparently induced lipid accumulation. White et al (2011) showed that the accumulation of oleic acid was correlated with an increase of astaxanthin esters when microalgae was grown under nitrogen starvation or high light intensity conditions.

Our observation (Table 1) showed that intracellular oleic acid concentration increased sharply (3.1 fold) in the cells exposed to stress conditions, though it is not the major species of fatty acid. Gustavs et al (2010) showed that oleic acid was the major fatty acid present in cysts. The differences in oleic acid content observed in this research could be triggered by differences in initial culture conditions (Damiani et al 2010). Recht et al (2012) showed that the de novo pathway induced most of the 18:1 and 16:0 at the

expense of the PUFAs 16:2, 16:4 and EPA. In this research, we also found out that PUFAs such as 16:2, 16:4, C18:3n6, ARA, and EPA decreased when *Symbiodinium* cells were cultivated under stress conditions. Solovchenko et al (2010) stated that EPA will be placed in chloroplast membrane under nutritional depletion; and cells are unable to resynthesize EPA and/or even keep the concentration of nutritional components constant.

Thus, we assumed that initial stresses would influence the photosystem. However, the relationship between photosynthetic efficiency and intracellular lipids concentration has not been widely studied in the previous studies (Petrou et al 2008; Gustavs et al 2010). Petrou et al (2008) showed that nutrient-depleted microalgae redirect energy from photosynthetic processes to maximize nutrient uptake upon nutrient addition, and tend to lead to a net decrease in the cell capacity to dissipate inert photochemical energy. This result suggests a means of photo-protection that the synthesis and the yield of neutral lipids may lead to be a sensitive protective mechanism for cells against environmental conditions (Courchesne et al 2009).

The environmental stress effect on carotenoid accumulation and lipid production was also observed in this research. We found out that there were significant changes in the pigment content of the *Symbiodinium* in [HL]-, [without N_2]-, and [without N_2-HL] cultures compared to the control one (Figure 3). It has been reported that under stress conditions, such as high light irradiance or nitrogen limitation, *Symbiodinium* form clusters of globules containing carotenoid at the cell centre (Peng et al 2012). Predominantly, intracellular carotenoid may vary as mono- and di-esters (Savage et al 2002) and as such suggests that carotenoid possibly plays a role as an antioxidant, which maybe inhibiting lipid peroxidation (Peng et al 2012). Our research data in Table 2 represent the linear correlative changes in intracellular lipid and carotenoid contents of *Symbiodinium*. The correlation between lipid and carotenoid contents under specific environmental stress conditions perfectly described the phenomena by providing the spearman correlation (r) from 0.844 to 0.983 and deterministic indexes (R^2) from 0.711 to 0.965. It was reasonable to assume that under specific culture condition the fatty acid metabolism may induce intracellular pigment accumulation in *Symbiodinium*. These results indicated that *Symbiodinium* must be a potential marine micro alga in the production of biofuel and carotenoid.

Table 2

Spearman (r) and deterministic (R^2) indices of total lipid contents and total carotenoid in the control, [HL], [without N_2], and [without N_2-HL] cultures

Parameter	*Control*	*HL*	*Without N_2*	*Without N_2-HL*
Equation	Y=1.104X+3.131	Y=0.332X+4.894	Y=0.352X+5.932	Y=0.866X+1.897
r	0.844	0.911	0.954	0.983
R^2	0.711	0.829	0.909	0.965

Note: HL refers to the cells under high light (450 µmol photons m^{-2} s^{-1} of continuous light); without N_2 refers to the cells under nitrogen starvation stress; without N_2-HL refers to the cells under high light (450 µmol photons m^{-2} s^{-1} of continuous light) and nitrogen starvation stress.

Figure 4 provides the linear correlation diagram of total lipid content and total carotenoid for all treatments. Our data represent the same trends of changes in total lipid and carotenoid contents. The relationship between lipid and carotenoid under stress conditions perfectly matched the above-mentioned point of view (Table 2) by providing correlation indexes (r) from 0.844 to 0.983. It was reasonable to assume that the fatty acid metabolism under specific conditions is inductive to pigment accumulation in *Symbiodinium*.

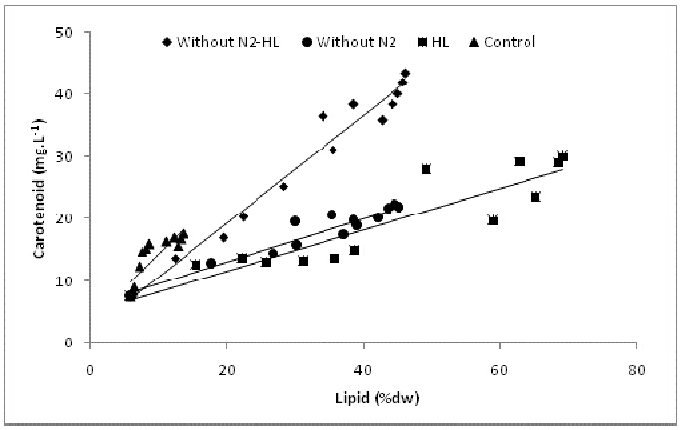

Figure 4. Correlation analysis between total lipid content and total carotenoid in *Symbiodinium* cells grown in the control, [HL], [without N_2], and [without N_2-HL] cultures.

Conclusions. The research described the suitable condition for intracellular lipid and carotenoid production by *Symbiodinium* grown under high light intensity and nitrogen depletion. In its initial culture condition, a highly positive correlation between intracellular carotenoid and lipid contents exposed the possibility of using *Symbiodinium* for the combined production of biofuel and carotenoid. Further studies might explore more specific culture conditions, such as the use of another potential environmental variable to provide a significant increase of lipid and carotenoid contents.

References

Ahmad A. L., Mat Yasin N. H., Derek C. J. C., Lim J. K., 2011 Microalgae as a sustainable energy source for biodiesel production: a review. Renewable and Sustainable Energy Reviews 15:584-593.

An M. L., Mou S. L., Zhang X. W., Zheng Z., Ye N. H., Wang D. S., Zhang W., Miao J. L., 2013 Expression of fatty acid desaturase genes and fatty acid accumulation in *Chlamydomonas* sp. ICE-L under salt stress. Bioresource Technology 149:77-83.

Atabani A. E., Silitonga A. S., Badruddin I. A., Mahlia T. M. I., Masjuki H. H., Mekhilef S., 2012 A comprehensive review on bio-diesel as an alternative energy resource and its characteristics. Renewable and Sustainable Energy Reviews 16:2070-2093.

Baker A. C., 2003 Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. Annual Review of Ecology and Systematics 34:661-689.

Chen H. K., Song S. N., Wang L. H., Mayfield A. B., Chen Y. J., Chen W. N., Chen C. S., 2015 A compartmental comparison of major lipid species in a coral-*Symbiodinium* endosymbiosis: evidence that the coral host regulates lipogenesis of its cytosolic lipid bodies. PLoS ONE 10(7):e132519.

Courchesne N. M., Parisien A., Wang B., Lan C. Q., 2009 Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. Journal of Biotechnology 141:31-41.

Damiani M. C., Popovich C. A., Constenla D., Leonardi P. I., 2010 Lipid analysis in *Haematococcus pluvialis* to assess its potential use as a biodiesel feedstock. Bioresource Technology 101:3801-3807.

Finney J. C., Pettay D. T., Sampayo E. M., Warner M. E., Oxenford H. A., LaJeunesse T. C., 2010 The relative significance of host-habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus *Symbiodinium*. Microbial Ecology 60:250-263.

Fitriyani W., Harpeni E., Muhaemin M., 2017 [Effect of light intensity on carotenoid, fucoxanthin, and phaeophytin pigments of zooxanthellae from soft coral *Zoanthus* sp. isolate]. Maspari Journal 9(2):121-130. [in Indonesian]

Gouveia L., Oliveira A. C., 2009 Microalgae as a raw material for biofuels production. Journal of Industrial Microbiology and Biotechnology 36:269-274.
Griffiths M. J., Harrison S. T. L., 2009 Lipid productivity as a key characteristic for choosing algal species for biodiesel production. Journal of Applied Phycology 21:493-507.
Gustavs L., Eggert A., Michalik D., Karsten U., 2010 Physiological and biochemical responses of green microalgae from different habitats to osmotic and matric stress. Protoplasma 243:3-14.
Hudaidah S., Muhaemin M., Rosdinar, 2015 Strategy of *Nannochloropsis* against environment starvation: population density and crude lipid contents. Maspari Journal 5(2):64-68.
Krueger T., Gates R. D., 2012 Cultivating endosymbionts - host environmental mimics support the survival of *Symbiodinium* C15 ex hospite. Journal of Experimental Marine Biology and Ecology 413:169-176.
Kuguru B., Achituv Y., Gruber D. F., Tchernov D., 2010 Photoacclimation mechanisms of corallimorpharians on coral reefs: photosynthetic parameters of zooxanthellae and host cellular responses to variation in irradiance. Journal of Experimental Marine Biology and Ecology 394:53-62.
Liang C., Zhai Y., Xu D., Ye N., Zhang X., Wang Y., Zhang W., Yu J., 2015 Correlation between lipid and carotenoid synthesis and photosynthetic capacity in *Haematococcus pluvialis* grown under high light and nitrogen deprivation stress. Grasas y Aceites 66(2):e077.
Mata T. M., Martins A. A., Caetano N. S., 2010 Microalgae for bio-diesel production and other applications: a review. Renewable and Sustainable Energy Reviews 14:217-232.
Moya A., Tambutte S., Tambutte E., Zoccola D., Caminiti N., Allemand D., 2006 Study of calcification during a daily cycle of the coral S*tylophora pistillata:* implications for 'light enhanced calcification'. Journal of Experimental Biology 209: 3413-3419.
Peng S. E., Chen C. S., Song Y. F., Huang H. T., Jiang P. L., Chen W. N., Fang L. S., Lee Y. C., 2012 Assessment of metabolic modulation in free-living versus endosymbiotic *Symbiodinium* using synchrotron radiation-based infrared microspectroscopy. Biology Letters 8:434-437.
Perez S. F., Cook C. B., Brooks W. R., 2001 The role of symbiotic dinoflagellates in the temperature induced bleaching response of the subtropical sea anemone *Aiptasia pallida*. Journal of Experimental Marine Biology and Ecology 256:1-14.
Petrou K., Doblin M. A., Smith R. A., Ralph P. J., Shelly K., Beardall J., 2008 State transitions and non-photochemical quenching during a nutrient-induced fluorescence transient in phosphorus-starved *Dunaliella tertiolecta*. Journal of Phycology 44:1204-1211.
Prartono T., Kawaroe M., Sari D. W., Augustine D., 2010 Fatty acid content of Indonesian aquatic microalgae. HAYATI Journal of Biosciences 17(4):196-200.
Prartono T., Kawaroe M., Katili V., 2013 Fatty acid composition of three diatom species *Skeletonema costatum, Thalassiosira* sp. and *Chaetoceros gracilis*. International Journal of Environment and Bioenergy 6(1):28-43.
Recht L., Zarka A., Boussiba S., 2012 Patterns of carbohydrate and fatty acid changes under nitrogen starvation in the microalgae *Haematococcus pluvialis* and *Nannochloropsis* sp. Applied Microbiology and Biotechnology 94:1495-1503.
Rosenberg J. N., Oyler G. A., Wilkinson L., Betenbaugh M. J., 2008 A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Current Opinion in Biotechnology 19:430-436.
Savage A. M., Trapido-Rosenthal H., Douglas A. E., 2002 On the functional significance of molecular variation in *Symbiodinium*, the symbiotic algae of Cnidaria: photosynthetic response to irradiance. Marine Ecology Progress Series 244:27-37.
Strychar K. B., Sammarco P. W., 2012 Effects of heat stress on phytopigments of zooxanthellae (*Symbiodinium* spp.) symbiotic with the corals *Acropora hyacinthus*, *Porites solida*, and *Favites complanata.* International Journal of Biology 4(1):3-19.

Solovchenko A., Merzlyak M. N., Khozin-Goldberg I., Cohen Z., Boussiba S., 2010 Coordinated carotenoid and lipid syntheses induced in *Parietochloris incisa* (Chlorophyta, Trebouxiophyceae) mutant deficient in Δ5 desaturase by nitrogen starvation and high light. Journal of Phycology 46:763-772.
Tang D., Hung C. C., Warnken K. W., Santschi P. H., 2000 The distribution of biogenic thiols in surface waters of Galveston Bay. Limnology and Oceanography 45(6):1289-1297.
Wang L. H., Liu Y. H., Ju Y. M., Hsiao Y. Y., Fang L. S., Chen C. S., 2008 Cell cycle propagation is driven by light-dark stimulation in a cultured symbiotic dinoflagellate isolated from corals. Coral Reefs 27:823-835.
Wang L. H., Lee H. H., Fang L. S., Mayfield A. B., Chen C. S., 2013 Fatty acid and phospholipid syntheses are prerequisites for the cell cycle of *Symbiodinium* and their endosymbiosis within sea anemones. PLoS ONE 8(8):e72486.
White S., Anandraj A., Bux F., 2011 PAM fluorometry as a tool to assess microalgal nutrient stress and monitor cellular neutral lipids. Bioresource Technology 102: 1675-1682.
Xu D., Gao Z., Li F., Fan X., Zhang X., Ye N., Mou S., Liang C., Li D., 2013 Detection and quantitation of lipid in the microalga *Tetraselmis subcordiformis* (Wille) Butcher with BODIPY 505/515 staining. Bioresource Technology 127:386-390.
Zamani N. P., Muhaemin M., 2016 [Spectrophotometer utilization as marine microalgae cells detector]. Maspari Journal 8(1):39-48. [in Indonesian]

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