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THE EFFECT OF ACIDIFICATION ON GROWTH AND PHOTOSYNTHESIS RATE OF SEAGRASS *Thalassia hemprichii* (Ehrenberg.) Ascherson

PENGARUH ASIDIFIKASI TERHADAP LAJU PERTUMBUHAN DAN FOTOSINTESIS LAMUN Thalassia hemprichii (Ehrenberg.) Ascherson

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ABSTRACT

Seagrass is a water plant that has flowers and ability to adapt to live and grow in the sea like a terrestrial plant. The survival of seagrass is greatly influenced by physical and chemical parameters of waters, such as pH, temperature, and salinity. The Intergovernmental Panel on Climate Change (IPCC) report by the end of 21st century, CO_2 in the atmosphere has doubled along with the industrial development. The increase in CO_2 in the atmosphere causes ocean acidification, it can change the chemical structure and decrease the pH of sea water. The low pH of sea water influences plant phisiology such as the inhibition of photosynthesis and growth. The purpose of this study is to examine the effect of pH on the growth and photosynthesis rate of seagrass *Thalassia hemprichii*. The study used Completely Randomized Design with 3 treatments control (8.10-8.50), medium pH (7.76-8.00) and low pH (7.50-7.75) in 5 replicates. The results showed that growth rate, photosynthetic rate and chlorophyll content has a bigger value on control treatment than the low pH treatment. The ANOVA test results were not significant for all treatment variables and had a negative impact on the survival of seagrass.

Keywords: acidification, chlorophyll, photosynthesis rate, *Thalassia hemprichii*

ABSTRAK

Lamun merupakan tanaman air yang memiliki bunga dan kemampuan beradaptasi untuk hidup dan tumbuh di laut seperti tanaman terestrial. Kelangsungan hidup lamun sangat dipengaruhi oleh parameter fisik dan kimia perairan, seperti pH, suhu, dan salinitas. Intergovernmental Panel on Climate Change (IPCC) melaporkan pada akhir abad 21, CO_2 di atmosfer selalu meningkat seiring dengan perkembangan industri. Peningkatan CO_2 di atmosfer menyebabkan terjadinya asidifikasi laut sehingga dapat mengubah struktur kimia dan pH air laut. Rendahnya pH air laut berpengaruh terhadap fisiologi tumbuhan seperti terhambatnya proses fotosintesis dan pertumbuhan. Tujuan penelitian ini adalah untuk menguji pengaruh pH terhadap pertumbuhan dan laju fotosintesis lamun Thalassia hemprichii. Penelitian ini menggunakan Rancangan Acak Lengkap dengan 3 perlakuan yaitu kontrol (8,10-8,50), pH sedang (7,76-8,00) dan pH rendah (7,50-7,75) dalam 5 ulangan. Hasil penelitian menunjukkan bahwa laju pertumbuhan, laju fotosintesis dan kandungan klorofil memiliki nilai lebih besar pada kontrol dibandingkan dengan perlakuan pada pH sedang dan rendah. Hasil uji ANOVA tidak signifikan untuk semua variabel perlakuan dan memiliki dampak negatif pada kelangsungan hidup lamun.

Kata kunci: *asidifikasi, klorofil, laju fotosintesis, Thalassia hemprichii*

I. INTRODUCTION

By the end of the 21st century it is estimated that carbon dioxide (CO_2) in the atmosphere has doubled along with industry development and increased usage of fossil energy such as coal, oil and gas (Chen & Millero, 1979; IPCC, 2007; Revelle & Suess, 1957). This condition can increase CO_2 in the atmosphere by nearly 40% and decrease the ocean pH by 0.3-0.4 units (Brouns, 1985; Caldeira & Wickett, 2003; Feely *et al*., 2004). Orr *et al.* (2005) modeled a decrease in pH by 7.949 in 2050 and 7.824 in 2100. The impact that can be generated from the increase of CO_2 in atmosphere is sea acidification (Turley, 2008). Acidification may cause chemical changes of carbonate in the relative proportions of CO_2, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) to shift the total dissolved inorganic carbon from CO_3^{2-}, to HCO_3^- (carbonic acid) and CO_2 (Ciais *et al.*, 2014). Water conditions at low pH can inhibit photosynthesis, inhibit growth, decrease immune response, witness fertilization and calcification of organisms (Kurihara *et al*., 2004; Brothers *et al*., 2016). Ow *et al*. (2015) revealed that acidification has an impact on photosynthesis and seagrass productivity.

Seagrass is a water plant that has flowers and adaptability to live and grow in the sea like a terrestrial plant (Kawaroe *et al*., 2016). The seagrass ecosystem has many important roles, such as habitat and shelter of various biota, nursery ground, spawning ground and stabilizing aquatic sediments (Guinotte & Fabry, 2008; Koch *et al*., 2012; Christianen *et al.*, 2013; Riniatsih & Endrawati, 2013). The seagrass ecosystem also plays an important role in the coastal carbon cycle (Oreska *et al*., 2017). One of seagrass species *T. hemprichii* known as dugong grass is a dominant species that can be found almost in all Indonesian waters and become key species of tropical seagrass in the Indo-Pacific region (Mukai, 1993).

Due to the important role of seagrass ecosystem makes several studies have been conducted to determine the response of acidification. Acidification can increase photosynthesis in *Zostera marina* and *Phyllospadix torreyi* (Beer *et al*., 2002; Koch *et al*., 2012), biomass changes and increased productivity of *T. testudinum*, *Halophila uninervis*, *Cymodocea serrulata* (Campbell & Fourqurean, 2013; Durako & Sackett, 1993; Ow *et al*., 2015). Based on several literature studies above, it is necessary for further study to determine the effect of acidification on growth rate and photosynthesis rate of *T. hemprichii* seagrass.

II. MATERIALS AND METHODS

The study was conducted from September to December 2017. *T. hemprichii* were taken in the Kepulauan Seribu while for transplantation and analysis of photosynthetic response was performed at the Marine Habitat Laboratory, Faculty of Fisheries and Marine Sciences, IPB University. Analysis of chlorophyll content was performed in the testing laboratory, Faculty of Agriculture, IPB University.

T. hemprichii was taken by digging the whole bud with the rhizome connected to the sediment. Afterwards the sample then was placed in a coolbox, then transferred to the aquarium at Marine Habitat Laboratory.

2.1. Research Design

The research was design using Completely Randomized Design (RAL). The aquarium was used as a container to grow 15 seagrasses for 3 treatments with 5 replications each. The treatment was pH in the range of 7.76-8.00 for medium level, low pH in the range of 7.50-7.75 and control. The position of the aquarium was placed randomly (Figure 1). The volume of sea water used is 29 L. The pH adjustment was performed by adding CO_3^{2-} 20.18 ppm for the low pH treatment and 19.79 ppm for the

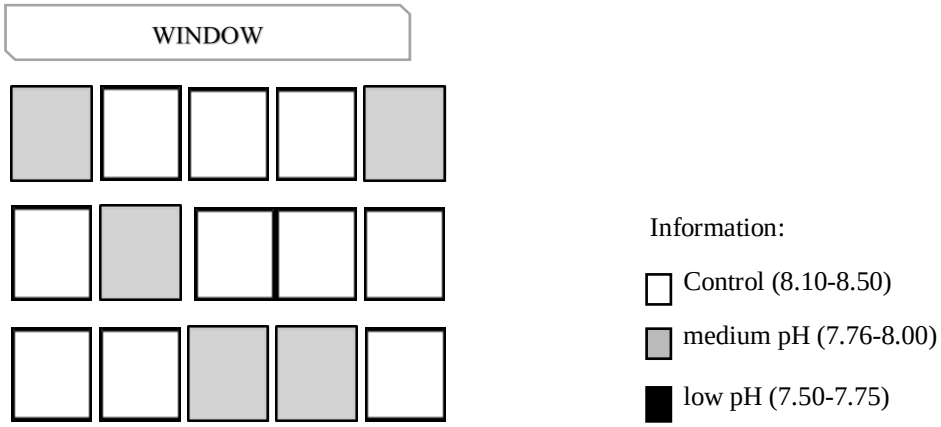

Figure 1. Experimental design of the study with two different pH range as treatment.

mean pH, whereas the controls were added 19.20 ppm. LED light (Light Emitting Diode) P 800K was mounted 30 cm above the aquarium surface. Lighting time was 12 hours light and 12 hours dark. Sea water was replaced every 2 weeks while NASA fertilizer is added to support seagrass growth every 1 week as much as 29 mL.

Physical chemical parameters are measured daily at 03.00 PM. Water quality parameters measured in insitu that includes temperature using thermometer, salinitt using refractometer, and pH using pH meter.

2.2. Growth Rate

Growth rate of seagrass were measured by leaves length at certain intervals, in the beginning and end of the observation. The formula for growth rate is as follows (Short & Coles, 2001):

$$P = \frac{P_t - P_0}{\Delta_t} \quad \text{.....(1)}$$

Information: P= Leaf growth rate (mm/day); P_t= Leaf length at final measurement (mm); P_0= Leaf length at initial measurement (mm); Δt= Time measurement interval (days).

2.3. Chlorophyll Content Analysis

Determination of chlorophyll content was administered on young adult leaves of each seagrass samples. The seagrass leaves was dried and then weighed. The sample then was added 90% acetone solution. Each tube was filled with solvent up to 6 ml then centrifuged at 4000 rpm for 12 minutes. All samples were frozen to prevent heat and light degradation. A total of 1 ml of supernatant was transferred into cuvette for spectrophotometry analysis at the wavelengths of 664.0 and 647.0 nm. Calculations of chlorophyll content (mg/g) are as follows (Granger & Izumi, 2001).

Chlorophyll a = 11.93 E_{664} - 1.93 E_{647} ....(2)

Chlorophyll b = 20.36 E_{647} - 4.68 E_{664} ....(3)

Information: E_{664}= Correction of absorbance, (absorbance at wavelength of 664 nm - absorbance at 750 nm).

2.4. Photosynthesis Rate

The seagrass leaves at each treatment were taken and dried before the measurement of photosynthesis rate. The measurement of photosynthesis rate were performed by LI-COR 6400XT Portable Photosynthesis System equipped with CO_2 injector and LED light source. The leaves of seagrass were put into the chamber for a few seconds. Observational data were analyzed by program arrangement in the form of light intensity response curve (PAR= Photo-synthesis Active Radiation) from 1-1200

μmol CO_2 m^{-2} s^{-1} as abscissa and net photosynthesis as its coordinates so it obtain the curve of seagrass photosynthetic growth rate on control and every treatment observed (Soleh, 2017).

ANOVA was applied to test significant differences between experimental treatment (carbonate addition (CO_3^{2-}) to decrease pH) in all response variables (growth rate, chlorophyll and photosynthesis rate) at value of P = 0.05 (Suliyanto, 2012).

III. RESULT AND DISCUSSION

3.1. Physics and Chemical Water Parameters

Seagrass survival is influenced by physical and chemical factors of water directly or indirectly. Physical and chemical water parameters are presented in Table 1. The pH value of waters at control ranged from 8.07 to 8.48. The pH range at pH treatment (7.76) is 7.75-7.83. The pH range at the pH treatment (7.58) is 7.57-7.65. The increase in pH is thought to be due to reduced CO_2 due to photosynthetic activity.

Table 1. Physical-chemical water parameters.

Parameters	Treatment of pH		
	Control	Medium	Low
pH	8.32	7.76	7.58
Temperature (˚C)	28.68	28.66	28.70
Salinity (psu)	32.48	32.89	32.60

The temperature range of the controls are 27.5-29.6 ˚C, for the medium pH treatment are 27.5-29.9 ˚C and for the low pH treatment are 27.4-29.6 ˚C. Based on the temperature in Table 1, the temperature of waters on the control and treatment of pH (7.76) and pH (7.58) is still within the optimum range for growth and photosynthesis on *T. hemprichii* which is 27-30˚C (Kondoy *et al*., 2014). Water temperatures that fall outside the normal range can still be tolerated by the seagrass plants but will disrupt the physiological process. The temperature range of 25-30˚C will increase photosynthesis along with rising temperatures. If the temperature of the waters exceeds the optimum limit of seagrass growth it can lead to stress, death and loss of genetic biodiversity due to the high respiratory process compared to photosynthesis (Chefaoui *et al*., 2018; Staehr & Borum, 2011).

The salinity of *T. hemprichii* in the controls is in the range of 30.4-34.4 psu. The pH treatment (7.76) is in the range of 30.6-34.8 psu while the pH treatment (7.58) is in the range of 30-35.2 psu. Based on the salinity value in Table 1 it shows that the salinity range in the control and treatment is still within the optimum salinity of 20-40 psu (Touchette & Burkholder, 2000). Salinity that exceed the optimum limit causes stress on seagrass, it can reduce chlorophyll content, nutrient absorpsion, decreased leaf growth rate, morphological changes, photosystem function and death (Kuo & Lin, 2010; Marin-Guirao *et al*., 2013). Based on Table 1 shows that temperature and salinity in the control and treatment of medium and low pH are at the optimum range for seagrass growth.

3.2. Growth Rate of *T. hemprichii*

Leaf length growth is the difference in leaves that grow within specified time intervals (30 days). Based on Figure 2, the average length of seagrass leaves in the control was 3.76 mm/day with the growth rate ranged from 2.07 to 4.63 mm/day. The average length of seagrass leaves in pH treatment (7.76) is 2.32 mm/day and growth rate is in the range of 0.33-6.33 mm/day. The pH treatment (7.58) has an average length of 2.21 mm/day with a hose value of 0.83-4.1 mm/day. The ANOVA test showed that the average leaf growth rate was not significantly different (P> 0.05) among treatments.

The growth rate of seagrass leaves is

directly proportional to the content of chlorophyll and the rate of photosynthesis. The low rate of leaf growth in the treatment is suspected because that the seagrass is still adapting to the new environment. Seagrass growth is influenced by internal factors such as physiology, metabolism and external factors such as nutrient content of the substrate fertility and marine environmental parameters (Alexandre *et al.*, 2012; Ow *et al*., 2015). These environmental factors affect the metabolism of carbon and nitrogen. Lack of nitrogen in the pH treatment (7.76) and pH (7.58) affects the formation of chlorophyll that interferes with the photosynthesis process and inhibits growth in seagrass leaves.

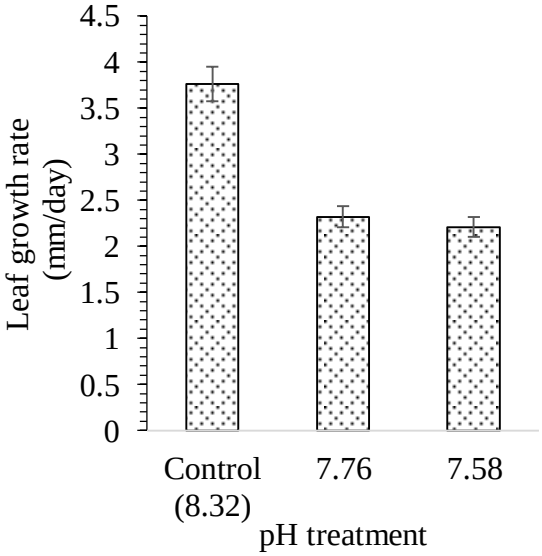

Figure 2. *T. hemprichii* leaf growth rate in control and treatment for 30 days.

3.3. Content of Chlorophyll *T. hemprichii*

Chlorophyll is a pigment owned by plants and plays a role in the process of photosynthesis. Chlorophyll absorbs and uses the energy of sunlight for the synthesis of oxygen (O_2) and carbohydrates of CO_2 and water. Chlorophyll content on seagrass tissue can affect the physiological response. Seagrass plants contain only 2 chlorophyll pigments namely chlorophyll a and chlorophyll b. The difference of the chlorophyll pigment lies in the absorbed wavelength. Based on Figure 3 the average content of chlorophyll a and b in the controls were 1.83 and 1.10 mg/g with a chlorophyll a in the range of 1.22-2.49 mg/g and chlorophyll b 0.76-1.47 mg/g. The pH treatment (7.76) average content of chlorophylls a and b are 1.36 and 0.83 mg/g with of each chlorophyll is in the range of 1.09-2.33 mg/g and 0.75- 0.83 mg/g. The pH treatment (7.58) has an average of chlorophyll a and b of 0.91 and 0.56 mg/g with of each ranging from 0 to 1.66 mg/g and 0-1.06 mg/g. The result of ANOVA analysis showed that average yield of chlorophyll content is not significant ($P > 0.05$).

The amount of chlorophyll in a plant will affect the photosynthesis and growth of seagrass. The low chlorophyll content of treatments especially in low and medium pH is seen from the color of the seagrass leaf form pale to black (Figure 4).

The amount of chlorophyll is determined by the availability of light, depth, type of seagrass, leaf age and nutrien (Wiginton & McMillan, 1979; Lee *et al*., 2007; Clores & Carandang, 2013). The color changes on the leaves of the seagrass are suspected caused by a stressful condition to the environment. Adaptation mechanisms on seagrass when stress will depend on the intensity, type and duration of the disturbance. When in a state of stress, the seagrass will lose the chlorophyll and carotenoid molecules so the leaf color will be different when the leaves are naturally aging (Duarte *et al*., 2012). The pheophytin reaction occurs when the plant is in an acidic pH. In acidic conditions, chlorophyll will be very easily degraded and will form peophytin due to the shift of two hydrogen (H) atoms (Dwidjoseputro, 1994). The pressure from H atoms causes the loss of Mg^{2+} ions which is the main components of chlorophyll so that the color of the leaves in the medium and low pH treatment are brown

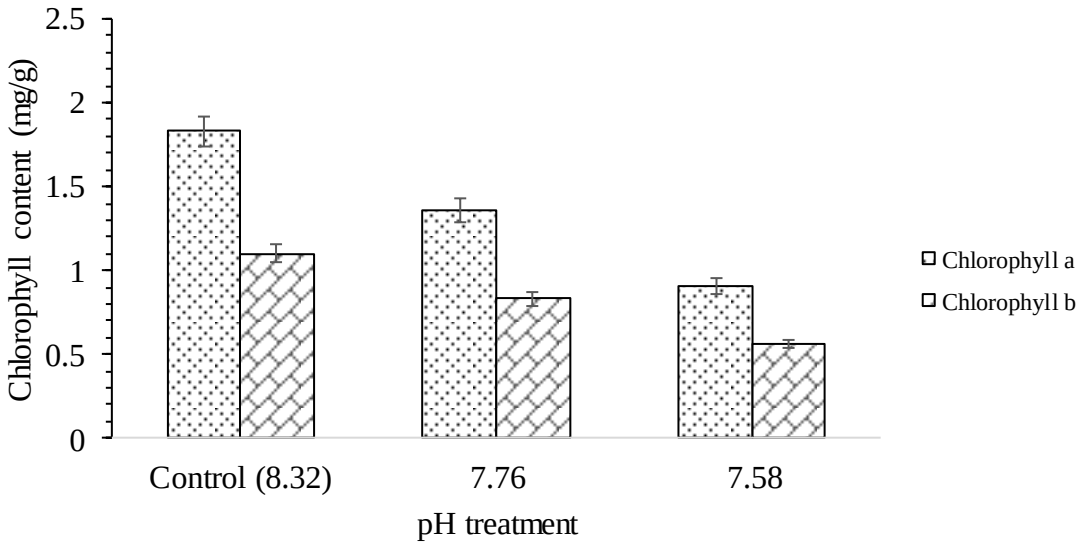

Figure 3. Chlorophyll content in control and treatment for 30 days.

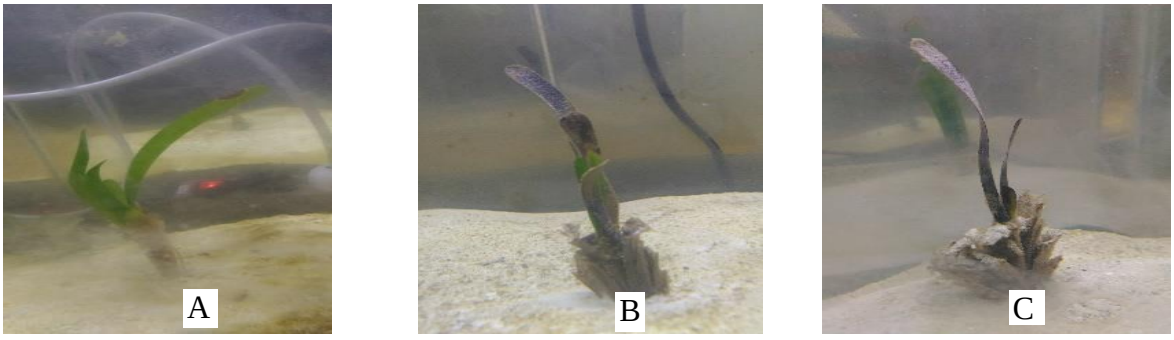

Figure 4. The condition and color of *T. hemprichii* leafs on control (A), pH 7.76 (B) and pH 7.58 (C) for 30 days.

(Figure 4). Loss of chlorophyll content will inhibit the process of growth and photosynthesis.

3.4. Photosynthetic Rate *T. hemprichii*

The mean value of the photo-synthetic rate (Figure 5) in the control of 19.11 µmol CO_2 m^{-2} s^{-1} is in the range of 15.62-21.39 µmol CO_2 $m^{-2}s^{-1}$. The mean value of photosynthesis rate in pH treatment (7.76) is 12.61 µmol CO_2 m^{-2} s^{-1} in the range 0-18.28 µmol CO_2 m-2 s-1 while at pH (7.58) the value is 12.31 µmol CO_2 m^{-2} s^{-1} and is in the range of 0-17.71 µmol CO2 m^{-2} s^{-1}. The ANOVA test showed an insignificant pH decrease (P> 0.05) against the mean rate of photosynthesis. Seagrass capacity in response to changes in aquatic environments primarily to pH depletion depends on the availability of nutrients and light.

Factors that influence the rate of photosynthesis in addition to light and nutrient composition are chlorophyll content and tissue age. The rate of photosynthesis in the control was higher when compared to the treatment at pH (7.76) and pH (7.58), this corresponds to the chlorophyll content in each treatment. The low chlorophyll content of medium and low pH treatments caused a low rate of photosynthesis in seagrass *T. hemprichii*. This is because chlorophyll plays an important role in the absorption of free CO_2 in the waters (Ariyati

et al., 2007). The availability of CO_2 in the waters affects the process of photosynthesis. Addition of CO_3^{2-} results in reduced CO_2 free in the waters and an increase in the amount of O_2 (Susana, 1988).

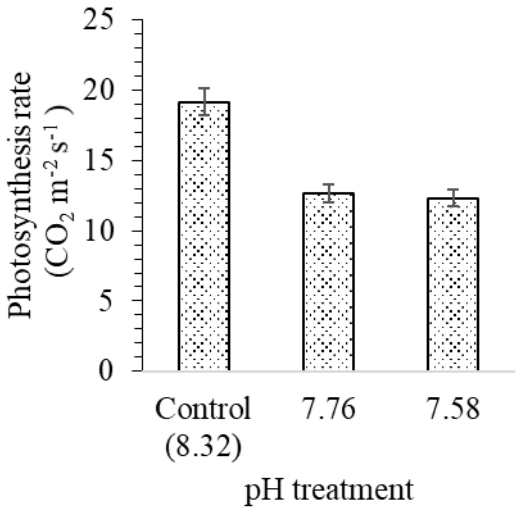

Figure 5. Photosynthetic rate of *T. hemprichii* in control and treatment for 30 days.

In the control treatment, seagrass will use HCO_3^- during photosynthesis which is abundant so that when photosynthesis takes place, seagrasses will use carbon from HCO_3^- to replace CO_2. The HCO_3 is used by extracellular dehydration of HCO_3^- into CO_2 through a carbonic anhydrase (CA) enzyme bound to the membrane (Uku *et al*., 2005).

IV. CONCLUSION

The ANOVA test were not significantly different among treatments, however, growth rates, chlorophyll content, and photosynthetic rate of *T. hemprichii* seagrasses had greater value on the control when compared with lower pH treatment. Acidification has negative effect on growth rate, chlorophyll content and photosynthesis rate of *T. hemprichii*.

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