Morphometric and growth responses of *Enhalus acoroides* seedlings under carbon dioxide enrichment: An experimental assessment

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Abstract. It is predicted that the concentration of carbon dioxide in the ocean will continue to increase. This phenomenon certainly has an impact on the sustainability of the marine ecosystem, including the seagrass ecosystem. This study aims to determine the effect of carbon dioxide on the morphometrics and growth of *E. acoroides* seedling. This study was an experimental study where the seeds from the fruit were grown in a controlled environment for two months. There are two treatments, first treatment with the addition of carbon dioxide and second treatment without the addition of carbon dioxide. The results of this study indicate that there is significant result from the two treatments given. Seagrass seeds that grow on treatment with carbon dioxide gas generally have shorter morphological characteristics as well as their growth.

Keywords: carbon dioxide; *E. acoroides*; growth; morphometric; seeds

1. Introduction

Concentration of carbon dioxide (CO\(_2\)) in the atmosphere perpetually increasing. Due to the increasing number of human activities, this phenomenon is occurred especially in utilizing of fossil fuels such as petroleum, natural gas and coal [1]. The increasing of carbon dioxide in the atmosphere affects the increasing of carbon dioxide in the oceans. The increasing of carbon dioxide in the oceans is potentially lower in pH or referred to as ocean acidification.

Process of ocean acidification is occurred due to the carbon dioxide that enters the sea is reacted to the air to form carbonic acid compounds. The more carbon dioxide enters the sea, the pH in the air will decrease. The pH of seawater is estimated will decrease from 8.2 before the industrial revolution to approximately 7.8 [2] The utilizing of fossil fuels perpetually in a long period of time is predicted to reduce the seawater pH and is estimated that in 2300 the average pH of sea air on the earth's surface will...
decrease to a maximum of 0.77 [3]. Estimated that the global pH value of seawater in 2100 will decrease to 7.5 [1].

Ocean acidification is potentially effects on the sustainability of marine organisms. The process of ocean acidification is due to the destruction of marine habitats, marine resource facts and change the function of marine ecosystems [4]. The majority of marine life are only able to tolerate pH values in the range 0.5 to 1.0 in a long time [5]. The low pH of seawater can effects on the physiology of marine life, such as photosynthesis, calcification and growth.

Seagrass is a flowering plant (angiosperms) which live submerged below sea level. Seagrass lives by forming ecosystems and has an important ecological role such as absorbing carbon and a source of food for marine biota [6-8]. Research related to seagrass response to ocean acidification has been implemented on several species such as Thalassia hemprichii, Zoostera polichlamys, Zoostera marina [9-11]. The research was conducted on mature seagrass plants. Little research has been conducted on the effect of ocean acidification on the physiology of seagrass seeds. The seeds have an important role in the development process of seagrass. E. acoroides is a seagrass which commonly found in the Indo-Pacific region [12]. This study aims to study a morphological and growth of E. acoroides seeds as response to carbon dioxide enrichment.

2. Material and methods

2.1. Seagrass fruit collect
Seagrass fruit used in this study is ripe seagrass. Seagrass fruit comes from the East Bintan coastal area. Furthermore, the seeds which taken from the seagrass is treated in a controlled environment for two months.

2.2. Design of experiment
Experiment was done in Marine Biology Laboratory. Each seagrass seed is grown in a glass of substrate (mud). Then each glass of seeds is put into a tub which has been filled with seawater. Each tub contains 30 glasses filled with seeds. One tub represents one treatment. There are two treatments in this study. The first treatment was treatment without giving carbon dioxide, this treatment acted as a control. The second treatment is treatment by giving 100 ml/week of liquid carbon dioxide.

2.3. Morphometric characteristic
The observations of the morphometric characteristics of E. acoroides seagrass seeds included measurements of leaf length, leaf width and root length. Measurements were conducted at the end of the study. Until the end of the study, the rhizome was not fully formed. Thus, the measurements were not taken.

2.4. Growth analysis
Growth observations were based on changes in the length of seagrass seed leaves. The growth rate was calculated by dividing the average leaf length during the observation by the length of time observed:

\[ P = \frac{L_t - L_0}{\Delta t} \]

Where:
- \( P \) = growth rates (mm/day)
- \( L_t \) = length of leaves in t-0
- \( L_0 \) = length of leaves in t-0
- \( \Delta t \) = interval time of observation

2.5. Statistical analysis
The research data were then analysed using Analysis of Variance (ANOVA) to determine whether the treatment effects on the morphology and growth of seagrass seeds.
3. Result and discussion

3.1. Morphometric characteristic
The experimental results for 60 days showed that the morphometric characters of seagrass treated under control conditions such as leaf length and leaf width had the larger than carbon dioxide treatment, while the root length of seagrass treated under carbon dioxide have the larger size than control (figure 1). The length of seagrass leaves under control treatment was in the range of 7.34 - 10.68 cm with an average leaf length of 8.75 cm, while the leaf length of seagrass treated with carbon dioxide was in the range of 4.74 - 6.46 cm with an average leaf length of 5.55 cm. Leaf width in the control treatment was in the range of 0.38 - 0.44 cm with an average value of 0.41 cm, while the leaf width of seagrass treated with carbon dioxide, was in the range of 0.35 - 0.4 cm with an average value of 0.4 cm. The root length in the control treatment was in the range 0.85 - 5.40 cm with an average value of 3.96 cm, while the root length was of seagrass treated with carbon dioxide, was in the range 2.70 - 6.20 cm with an average value of 5.21 cm. ANOVA test results showed that the morphological characteristics of the two treatments had significantly different values (p < 0.05).

Giving carbon dioxide gas with high concentrations to the seagrass growing media was affected to the formation of more carbonic acid (HCO$_3$) compounds. This is due to the large amount of carbon dioxide which reacts with water [13]. The high level of carbonic acid compounds has an impact on the low pH value of the waters. pH value of waters in CO$_2$ treatment equal to 6.7, this indicates the occurrence of an acidification process. Ocean acidification can inhibit the physiological processes that occur in seagrass [14]. Resulted of research show that the Posidonia oceanica seagrass which lives at low pH has a smaller size than the seagrass which lives in a normal pH conditions [15]. Another study showed that Posidonia oceanica seagrass seeds had morphological characteristics that were not significantly different between live seagrass seeds treated with carbon dioxide and control treatment [16]. pH value under 8 caused the concentration of chlorophyll and photosynthesis rate lower than normal pH [17]. In this study, E. acoroides seagrass seeds which were given carbon dioxide treatment had smaller morphological characteristics than the control treatment. This shows that E. acoroides seagrass seeds are still able to grow in response to environmental changes. Seagrass is able to respond to all changes that occur in the environment to experience stress [18].

![Figure 1. Morphometric characteristics of E.acoroides seagrass seeds (a) leaf length (b) leaf width (c) root length on control treatment (black) and treatment of carbon dioxide (grey).](image-url)
3.2. Leaf length growth of seeds

Seagrass seed growth observed in this study included leaf length growth. Leaf length growth observations are the easiest to observe and not interfere the life process of seagrass seeds. Based on the observations, the growth of seagrass seeds in the control treatment had faster growth than the growth of seagrass seeds which given by carbon dioxide gas (figure 2).

![Figure 2. Leaf length growth of *E. acoroides* seagrass seeds (blue line: CO\(_2\); red line: Non CO\(_2\)).](image)

The leaf growth rate of the seeds by control treatment was 1.50 mm/day, while the treatment with carbon dioxide gave the leaves a growth rate of 0.99 mm/day. The growth of seagrass seeds in this study had significantly different values for each treatment (P < 0.05). The slow growth of seagrass seeds in the treatment of carbon dioxide gas is due to the low pH value of the waters which causes the ocean acidification process. Acidification has an impact on the low content of photosynthetic pigments [19]. The content of photosynthetic pigments is related to the processes of photosynthesis, growth and physiology in seagrass [20]. Another research explains by that the lower the pH of the waters has an impact on the lower rate of photosynthesis in seagrass *Cymodocea rotundata* [17]. The low rate of photosynthesis has an impact on the low rate of leaf growth. The growth of seagrass seeds is also influenced by the condition of the aquatic environment which is influenced by the size of the seeds [21].

4. Conclusion

Giving carbon dioxide gas to the water caused a decrease in pH which affects the smaller morphology of the seagrass than the control treatment. Giving carbon dioxide gas also caused inhibit growth of seagrass seeds.

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