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METABOLISM CHARACTERISTICS OF SEAGRASS *HALOPHILA SPINULOSA*: CARBON DIOXIDE ABSORPTION RATE IN PHOTOSYNTHESIS AND OXYGEN USE IN RESPIRATION

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ABSTRACT

One of the roles of seagrass is as a carbon sink so its presence in the waters is very important. Each species of seagrass has a different character related to photosynthesis and respiration. The study was conducted to analyze the rate of carbon dioxide uptake in photosynthesis and the rate of oxygen use in respiration of *Halophila spinulosa* seagrass. The research was conducted in July 2022 in Puntondo Waters, Takalar Regency, South Sulawesi. Seagrasses were taken whole from waters 1 meter deep and then cleaned. The method used to analyze carbondioxide uptake and oxygen use was oxygen changes using clear and dark bottles. Each one shoot of *H. spinulos* was incubated using a clear and dark glass bottle with a volume of 270 ml. Incubation was carried out at 09.20 WITA (Middle Indonesian Time) - 12.20 WITA at a depth of 50, 100 and 150 cm with 5 replicate bottles at each depth. In addition, incubation of seawater (containing phytoplankton) was also carried out with 5 replicate bottles as a correction. At the beginning of incubation, the dissolved oxygen concentration in the water was measured. After incubation, oxygen was measured in clear and dark bottles. Seagrass leaves that have been used were scanned and analyzed using Image-J software to determine the extent. Seagrass leaves were dried in the oven, then weighed to determine the biomass. The results showed that carbon dioxide uptake per shoot ranged from 0.53 to 0.84 mgCO_2/shoot/hour, per biomass 12.98-28.34 mgCO_2/g/hour, and per leaf area 0.02-0.05 mgCO_2/cm^2/hour. The highest rate of carbon dioxide uptake was at a depth of 150 cm, both carbon uptake per shoot, biomass and leaf area. In contrast to the absorption of carbon dioxide, the rate of use of oxygen for respiration is highest at a depth of 50 cm. The use of oxygen per shoot ranged from 0.078-0.157 mgO_2/shoot/hour, per leaf biomass 2.227-3.091 mgO_2/g/hour and per leaf area 0.006-0.010 mgO_2/cm^2/hour.

Keywords: Carbon dioxide absorption, oxygen consumption, global warming, seagrass *Halophila spinulosa*, Puntondo Takalar.

INTRODUCTION

Seagrass as one of the living marine plants is widely distributed in the world (McKenzie, et al., 2020), ranging from tropical to temperate waters except for polar waters. In Indonesian waters, the area of validated seagrass beds reaches 293,464 - 875,967 hectares (Sjafrie et al. 2018), ranging from the coastal waters of the mainland to thousands of small islands. Seagrass vegetation can form a large growing area to form a field so it is known as a seagrass meadow. However, in some locations it is often found growing only in small groups and spread over several areas.

As marine plants, seagrasses have a major role as primary producers (Koopmans, et al., 2020), occupying the first link in the food chain in coastal waters, along with other marine plants. One of the main characteristics of primary producers is the ability to carry out photosynthesis, which converts inorganic materials into organic matter. In simple terms, the process of photosynthesis utilizes carbon dioxide and water and converts them into carbohydrates and oxygen by utilizing energy from sunlight. Seagrass grows in water so that it utilizes carbon dioxide in the water column. In addition, under certain conditions seagrass can also use bicarbonate as a carbon source (Larkum et al., 2006; Purvaja, et al. 2020).

Although seagrasses indirectly utilize carbon sources from the air, they also play a role in reducing carbon in the air. One of the carbon sources in the waters is carbon from the air which can diffuse into the water column through the water surface. Thus, the presence of seagrass vegetation in coastal waters and islands also plays a role in reducing carbon which is the cause of global warming (de los Santos et al., 2020).

There are 15 species of seagrass in Indonesia, namely *Enhalus acoroides, Cymodocea rotundata, C. serrulata, Halophila decipiens, H. ovalis, H. minor, H. spinulosa, Haludole pinifolia, Halodule uninervis, Syringodium isoetifolium, Thalassia hemprichii,* and *Thalassodendron ciliatum*. Three other species, namely *Halophila sulawesii* which is a relatively new seagrass species published by Kuo (2007), *Halophila beccarii* only found its herbarium without a clear explanation, and *Ruppia maritima* which is also only found in the form of herbarium collections from Ancol-Jakarta and Pasir Putih in East Java. (Sjafrie et al., 2018). Among these 15 species of seagrass found, 3 species have a very limited distribution, i.e,. *Halophila decipiens, H. spinulosa* and *Thalassodendron ciliatum*.

The carbon absorption ability of seagrass species with a wide distribution has been studied (Mashoreng, et al. 2020), while seagrass with a limited distribution has only been studied in the *Thalassodendron ciliatum* species (Mashoreng et al. 2019), but the other two species have not been studied, including *Halophila spinulosa*. This species of seagrass in South Sulawesi Province is only found in Puntondo waters, Takalar regency (Priosambodo, 2007; Yasir and Moore, 2020). Meanwhile in Indonesia, the data collected by Sjafrie et al. (2018) from 366 sampling locations spread throughout Indonesia, the species of *H. spinulosa* was only found in 5 locations. This research is intended to estimate the ability of this species to absorb carbon dioxide in the photosynthesis process and to use oxygen in the respiration process so that it will complete data on the carbon absorption ability of various species of seagrass in Indonesia.

MATERIALS AND METHODS

Seagrass Samples

Seagrass *Halophila spinulosa* samples were collected from the subtidal zone in Puntondo Waters, Takalar Regency, South Sulawesi Province (5º35'32.29" South Latitude and 119º29'3.43" East Longitude) (Figure 1). The sampling location for *H. spinulosa* seagrass has several characteristics (Table 1).

Figure 1. Map of the Study Sites

Table 1. Characteristics of seagrass sampling locations *H. spinulosa*

Environmental Parameter	Range	Average
Temperature (ºC)	28-31	30
Salinity (º/ₒₒ)	29-35	31
Turbidity (NTU)	0.43-1.49	0.57
Current Velocity (m/s)	0.030-0.097	0.073
Wave Height (cm)	6.1-7.6	6.6
Water Depth (cm)	75 – 100	90
Sediment Grain Size (mm)	0,242-0,488	0,363

The method used to determine the rate of absorption of carbon dioxide in the photosynthesis process and the rate of oxygen use in the respiration process of seagrass *H. spinulosa* was the method of changing oxygen. The equipment used was a clear bottle and a dark bottle with the research procedure following Mashoreng et al. (2019; 2020). Seagrass species *H. spinulosa* were collected from the subtidal area of Puntondo Beach, Takalar Regency, South Sulawesi Province at a depth of 100 cm.

Incubation of clear bottles and dark bottles was carried out for 3 hours (Mateo et al., 2001), from 09.20-12.20 WITA (Middle Indonesian Time) at a depth of 50 cm, 100 cm and 150 cm below the water surface with 5 repetitions each. Determination of the depth was based on the distribution of seagrass species *H. spinulosa* at the research site, which is at a depth of 0.75-1.25 m (Yasir and Moore, 2020). At each depth, 5 bottles of seawater (containing plankton) were also incubated as a correction factor. At the beginning of incubation, measurements of dissolved oxygen in the water as initial oxygen were carried out. Final oxygen measurements were made on water from clear and dark bottles after the incubation period ended. Oxygen measurement was carried out using the Winkler titration method (Parsons et al., 1984).

Dissolved oxygen values and primary productivity were obtained using the APHA formula (mashoreng et al., 2019). However, the photosynthetic quotient value for seagrass in this formula is used 1.25 and for phytoplankton it is used 1.20 (Kaladharan and Raj, 1989). The value of primary productivity was converted into the value of carbon dioxide absorbed by the seagrass. Every 1 gram of carbon produced used 3.67 grams of co2 (equivalent).

All seagrass leaves that have been used in bottles were measured in area. Seagrass leaf area was used to determine the ability of carbon absorption per leaf area. The method of measuring seagrass leaf area was carried out by analysing photos of seagrass leaves using Image J Software as used by Mashoreng et al. (2019). Furthermore, the seagrass leaves were dried using an oven at 40°c for 72 hours, then weighed using a digital scale with an accuracy of 0.001 gram to determine the biomass. The carbon uptake value was calculated by dividing the amount of CO_2 (equivalent) used by the incubation time, either per shoot, biomass or leaf area.

The rate of carbon dioxide absorption and oxygen utilization based on shoots was multiplied by the seagrass density value to estimate the rate of carbon dioxide absorption and oxygen use per unit area. Seagrass density was observed using plots measuring 50cm x 50cm, placed parallel to the coast, at a depth of 50cm, 100cm and 150cm, respectively. The number of plot repetitions at each of these depths was 5 plots. In addition to seagrass density, the plots also observed the percentage of seagrass cover. Only the density and cover of the *H. spinulosa* species were observed.

RESULTS AND DISCUSSION

The carbon dioxide absorbed by the seagrass *H. spinulosa* in the photosynthesis process showed that for the category per seagrass shoot, it was around 0.53-0.84 $mgCO_2$/shoot/hour; per leaf biomass ranged from 12.98-28.34 $mgCO_2$/g/hour; and per leaf area ranging from 0.02-0.05 $mgCO_2/cm^2$/hour. The results of the analysis of variance showed that the rate of carbon dioxide uptake between depths for all categories was significantly different ($P<0.05$). The highest rate of carbon dioxide uptake in all of these categories was found at a depth of 150 cm (Table 2). A high rate of carbon dioxide absorption at a depth of 150 meters was also found in the seagrass species *Thalassodendron ciliatum* in the Panrangluhu waters of Bulukumba Regency (Mashoreng et al. 2019a), but it was different from the *Thalassia hemprichii* species in the Gusung Bonebatang Waters, Makassar City, the highest at a depth of 200cm (Mashoreng et al. al. 2019b). When compared to several other species of seagrass in the waters of Panrangluhu and Gusung Bonebatang (Mashoreng et al. 2019a; 2020) in the per shoot category, *H. spinulosa* seagrass has a higher carbon dioxide uptake rate than *T. hemprichii*, *Cymodocea rotundata*, *Halophila ovalis* and *Halophila minor*, but lower than *T. ciliatum*, *Enhalus acoroides* and *Halodule uninervis*. In the category of per leaf biomass, the carbon dioxide uptake rate of *H. spinulosa* was higher than other species, except for *T. ciliatum* and *H. uninervis*. Meanwhile, in the leaf area category, the carbon uptake rate of *H. spinulosa* was higher than other species except *T. ciliatum*

Table 2. CO_2 uptake rate (equivalent) of seagrass *H. spinulosa* based on stand, biomass and leaf area

Rate of Carbondioxide Uptake	Depth (cm)		
	50 cm	100 cm	150 cm
Per shoot ($mgCO_2$/shoot/h)	$0.53{\pm}0.10^a$	$0.72{\pm}0.02^a$	$0{,}84{\pm}0{,}03^b$
Per leaf biomass ($mgCO_2$/g/h)	$12.98{\pm}3.28^a$	$15.94{\pm}1.59^{ab}$	$28{,}34{\pm}1{,}77^b$
Per leaf area ($mgCO_2/cm^2$/h)	$0.022{\pm}0.004^a$	$0.028{\pm}0.006^a$	$0{,}053{\pm}0{,}004^b$

Note: Different letters behind numbers in the same row indicate significantly different values (P<0,05)

The phenomenon of photoinhibition may be the cause of the high absorption of carbon dioxide at a depth of 150cm compared to a depth of 50m and 100m which have higher light intensity. High light intensity and temperature are obstacles to the physiological processes of seagrasses that grow in the intertidal zone in the tropics (Saewong et al. 2022). In conditions of excessive light or exceeding their optimal needs, seagrasses may become stressed so that it affects photosynthetic activity (Wuthirak et al., 2016; Phandee and Buapet, 2018).

The high rate of absorption of carbon dioxide at a depth of 150cm has not been able to be determined, that the photosynthesis process of seagrass *H. spinulosa* occurs optimally at that depth. This is because in this study there were no experiments at a depth of more than 150cm. Determination of incubation depth is based on the distribution of seagrass species *H. spinulosa* in Puntondo waters. On the other hand, the density of seagrass *H. spinulosa* also varies between depths and is highest at a depth of 150cm. The results of the analysis of variance showed that the density of seagrass *H. spinulosa* at a depth of 50cm and 100cm was not significantly different (P>0.05), but was lower than the density of seagrass at a depth of 100cm (P<0.05) (Figure 2a). Different things were shown by the percentage of seagrass cover which did not show any significant difference between depths (P>0.05) (Figure 2b). The relationship between density and percent cover is not always linear. One of the reasons is the possibility of seagrass with high density piling up so that some seagrass leaves are shaded by other seagrass leaves. Thus, the increase in density is not linear with the increase in cover. This condition was also found in the relationship between percent cover and biomass (Mallombassi et al. 2020).

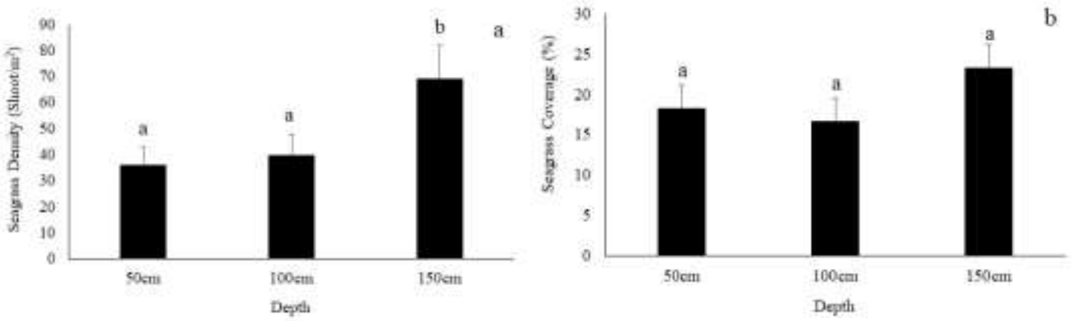

Figure 2. Condition of Seagrass *H. spinulosa* in Puntondo. (a) Seagrass density, and (b) Seagrass coverage

The rate of oxygen use for respiration per shoot ranged from 0.078-0.157 mgO_2/shoot/hour, per leaf biomass ranged from 2.227-3.091 mgO_2/g/hour and per leaf area ranged from 0.006-0.010 mgO_2/cm^2/hour. The oxygen utilization rate showed a significant difference between depths (P<0.05). In contrast to the rate of carbon uptake, the rate of oxygen utilization was found to be higher at a depth of 50cm than at a depth of 100cm and 150cm for all categories (Table 3). The high light intensity on the surface causes the water temperature to rise. The deeper the water, the temperature tends to decrease. In the optimal temperature range for respiration, an increase in temperature will increase the respiration rate, but if the temperature exceeds this range, the respiration rate will decrease. Several species of seagrass are often found in the upper part of the intertidal zone. Although high light intensity can inhibit the rate of respiration, it can quickly recover in the afternoon when light intensity begins to decrease. This was experienced by *H. ovalis* (Beer, et al. 2006). Seagrass respiration rate is influenced by internal and external factors. Internal factors include genetics and plant age, while external factors include temperature and oxygen availability in the waters (Lamit and Tanaka, 2021; Juska and Berg, 2022; Ward et al. 2022).

Referring to the density data of *H. spinulosa* seagrass, the rate of carbon dioxide uptake and oxygen use per unit area in Puntondo waters can be estimated. The rate of absorption of carbon dioxide in photosynthesis ranges from 19.0 to 58.4 $mgCO_2/m^2$/hour, while the rate of oxygen use in respiration ranges from 4.3 to 5.6 mgO_2/m^2/hour (Figure 3). The highest carbon dioxide absorption rate was found at a depth of 150cm, which was 58.4 $mgCO_2/m^2$/hour. This value is 2 times higher than at a depth of 100cm and 3 times higher than at a depth of 50cm. The rate of oxygen utilization was not significantly different between depths. Globally, the contribution of carbon dioxide

Table 3. Consumption of O_2 by seagrass *H. spinulosa* based on shoots, biomass and leaf area

Use of Oxygen	Depth (cm)		
	50 cm	100 cm	150 cm
Per shoot (mgO_2/shoot/h)	0.157±0.029^a	0.108±0.021^b	0.078±0.015^b
Per leaf biomass (mgO_2/g/h)	3.091±0.283^a	2.302±0.114^{ab}	2.227±0.111^b
Per leaf area (mgO_2/cm^2/h)	0.010±0.001^a	0.008±0.000^{ab}	0.006±0.001^b

Note: Different letters behind numbers in the same row indicate significantly different values (P<0,05) absorption of *H. spinulosa* species in Indonesia is relatively small due to its limited distribution, but information related to this right is needed to complete data on the rate of carbon dioxide absorption for all species of seagrass in Indonesia.

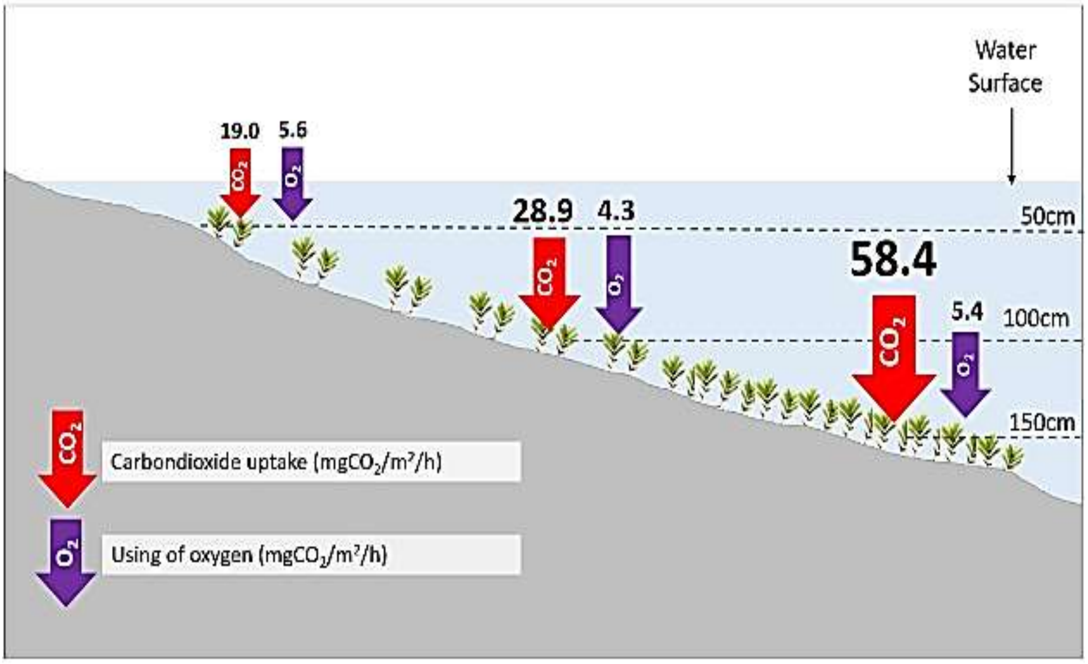

Figure 3. Rate of Carbon dioxide uptake (red) and using of oxygen (purple) per area (m^2).

CONCLUSION

The highest rate of carbon dioxide absorption in the photosynthesis process by seagrass *H. spinulosa* occurred at a depth of 150cm. However, it is not certain whether at that depth the photosynthesis process has reached optimal because there is no observation at a depth of more than 150cm. In the respiration process, the opposite phenomenon was found, namely the highest oxygen utilization rate at a depth of 50cm.

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