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Leaf area and stomatal density of *Rhizophora mucronata* Poir. under high and low light environment of Nature Tourism Park Angke-Kapuk, Jakarta, Indonesia

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ABSTRAK

Setiap jenis mangrove memiliki karakteristik stomata yang spesifik, salah satunya ialah kerapatan stomata. Kerapatan stomata ini berbanding lurus laju evaporasi, transpirasi, dan fotosintesis yang merupakan bentuk adaptasi mangrove terhadap lingkungannya. Salah satu faktor eksternal yang mempengaruhi kerapatan stomata mangrove adalah intensitas cahaya yang dapat bervariasi akibat tutupan tajuk. Penelitian ini bertujuan untuk membandingkan kerapatan stomata Rhizophora mucronata *Poir. sebagai jenis mangrove paling dominan di Taman Wisata Alam Angke-Kapuk. Lokasi tersebut merupakan kawasan konservasi mangrove yang telah mengalami banyak tekanan lingkungan terutama akibat pertambahan penduduk dan konversi lahan menjadi pemukiman. Penelitian ini menggunakan metode deskriptif dengan teknik sampling jalur transek pada lokasi yang mewakili tajuk yang sangat terbuka dan sangat tertutup yang ditentukan secara purposive sampling. Pengambilan sampel stomata dilakukan dengan cara mengoleskan cat kuku transparan pada permukaan bawah daun lalu menutupnya dengan selotip. Selotip tersebut diamati menggunakan mikroskop, dan hasilnya menunjukkan bahwa Rhizophora mucronata* Poir. *memiliki kerapatan stomata yang rendah. Hasil uji parametrik independent sample t-test menunjukkan bahwa kerapatan stomata Rhizophora mucronata tidak berbeda nyata pada tutupan tajuk yang sangat terbuka dengan sangat tertutup.*

Kata kunci: Kerapatan Stomata, Mangrove, Transek Garis, Transpirasi.

ABSTRACT

Each type of mangrove has specific stomatal characteristics, one of which is stomatal density. This stomatal density is directly proportional to the rate of evaporation, transpiration, and photosynthesis which is a form of mangrove adaptation to its environment. One of the external factors affecting stomatal density is light intensity which can vary due to canopy cover. This study aims to compare the stomatal density of Rhizophora mucronata Poir. in the Angke-Kapuk area, Jakarta, Indonesia. The location is a mangrove conservation area that has experienced a lot of environmental pressure, especially due to population growth and land conversion into settlements. Descriptive methods was used with transect sampling techniques at locations that represent very open and very closed canopies determined by purposive sampling. Stomatal sampling was carried out by applying a transparent nail polish on the lower surface of the leaf and then covering it with tape. The tape was observed using a microscope, and showed that Rhizophora mucronata Poir. has a low stomatal density. The results of the parametric independent sample t-test showed that the stomatal density of Rhizophora mucronata was not significantly different between very open and very closed canopy cover.

Keywords: Line Transect, Mangrove, Stomatal Density, Transpiration.

INTRODUCTION

Mangroves are tropical coastal vegetation that grow in tidal areas, living at the dynamic interface between land and sea, and have strong adaptability to extreme habitats (Alongi, D. M., 2018; Putra & Gumilang, 2019; Deng, H et al., 2021). The adaptability of mangroves includes tolerance to high salinity, low air humidity, low oxygen, and high temperatures due to direct sunlight exposure. One of the things that supports this adaptation is that mangroves have a leaf structure that can prevent excessive water loss. The anatomical structure of mangrove leaf physiology has a thick, waxy cuticle and hidden stomatal morphology (Fitriah et al., 2013; Dissanayake et al., 2018; & Marantika, Hiariej, & Sahertian, 2021).

Naskar et al. (2020) said that stomata play a role in the process of respiration, photosynthesis, and transpiration of mangrove plants to meet energy needs and water availability in cells. These three metabolic processes are influenced by external factors, such as water availability, wind speed, humidity, temperature, and light intensity. According to the results of research Wahyuningsih (2006), plants exposed to light with high intensity have stomata with a smaller size and a large number compared to those growing in shaded and humid places. This is in line with Batos et al. (2010) that leaves exposed to sunlight in open canopies have a higher stomatal density than leaves with shaded canopies. The level of stomatal density is generally directly proportional to the leaf area. This means that the greater the leaf area, the denser the stomata on the leaf.

Leaf area in mangroves that live in habitats exposed to direct light is different from shaded habitats. The leaf area in areas exposed to sunlight is greater than shaded areas. This is due to the impact of solar radiation which triggers the division of epidermal cells and stomata so that the photosynthetic process can be optimized (Batos et al, 2010; Haryanti, 2010; & Atmaja, 2011). However, Fathayati (2017) states that plants that grow in open canopy areas, including several types of mangroves, have adaptations like reeds. These plants have a narrower leaf area for evaporation efficiency, so they do not lose excessive water. The differences in adaptation patterns of stomatal density and leaf area of mangroves encourage further research on one type of mangrove that can grow in open and shaded canopies at the same time.

DKI Jakarta as an urban city has a mangrove conservation area that is packaged as a tourist attraction. One of them is the Angke-Kapuk Nature Tourism Park which is managed by the BKSDA DKI Jakarta. The 95.5 Ha area is the closest area to settlements but is still affected by tides, brackish water habitat, and is inhabited by endemic birds and long-tailed monkeys, which means it is still in wild nature (Suraji et al, 2015; Kusumahadi, K. S et al, 2020; & Febriyanto, 2020)

The most dominant mangrove species in Angke-Kapuk Nature Tourism Park is *Rhizophora mucronata* Poir. (Rumanta, 2019; Kusumahadi, K.S., Yusuf, A., Maulana, R. G., 2020). This type is evenly distributed throughout the ecotourism area with sparse-medium mangrove density criteria, including sparse mangroves 0.94 Ha, moderate mangroves 0.25 Ha, and dense mangroves that dominate up to 48.56 Ha. The distribution

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stretches from the entrance gate of the ecotourism with a shaded canopy to approaching the Sedyatmo toll access with an open canopy (Sofian et al., 2019; & Sentosa, B., Agus, S.B., & Arhatin, R. E., 2024). This condition is unique because according to Giesen et al. (2006) *R. mucronata* Poir. is the only mangrove species that is included in the highly exposed mangrove category with very high sun exposure. In fact, in Jakarta, including our study area, *R. mucronata* Poir. can live in places that are shaded by buildings, toll roads, and canopies of other plant species which cause low light intensity.

It is interesting to understand the adaptation of *R. mucronata* Poir. under differing light intensity? Do the leaves of *R. mucronata* Poir. Differ in their adaptation patterns in variable places? Is there a difference in stomatal density of *R. mucronata* Poir. in open and shaded canopies? And what is the relationship between stomatal density and leaf area of *R. mucronata* Poir. in places that have extreme differences in light intensity. The result will further enable us to understand the adaptation of *Rhizophora mucronata*, especially on life stages influenced by canopy cover. Furthermore, this research may contribute to the management and conservation of *R. mucronata* conservation in various types of habitats in Jakarta.

METHODOLOGY

The research method used is a descriptive method. Observations were made by determining areas with open and shaded canopies using purposive sampling with a lux meter measuring instrument. After getting significant different areas of light intensity, mangrove sampling was carried out using the line transect method. The difference in light intensity is representative of one research station.

Studies sites and species sampling

The research was conducted at Nature Tourism Park Angke-Kapuk, where we collected *R. mucronata* Poir.'s leaves in two significant different light intensities. Leaves samples observed and calculated at Plant Physiology Laboratory, Faculty of Mathematics and Natural Sciences, State University of Jakarta (UNJ), Indonesia from December 2020 to February 2021.

FIGURE 1. Research Location of Mangrove Ecotourism at Pantai Indah Kapuk with scale 1:24.000 (Febriyanto, 2020 & Google Maps).

The sampling technique used in this study is a line transect at a location that represents an open and shaded canopy as a research station (Alhani et al., 2015). Each

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station made a path with a length of 50 meters and a width of 20 meters. The path was made of 5 plots measuring 10 x 10 meters with intermittent location as a sampling location (Mansur et al., 2011). From each plot, 5 individuals/trees of *R. mucronata* Poir. with similar heights were selected by purposive sampling. From each individual, one leaf with the same relative area (approximately 25 cm^2) was taken so that 5 leaflets of *R. mucronata* Poir. were collected per sampling plot. Sampling was carried out at Nature Tourism Park Angke-Kapuk at 09.00 - 12.00 WIB with consideration to maintain the stability of the temperature of the leaves of *R. mucronata* Poir.

The number of samples taken from station 1 as a representative of the open canopy is 25 leaves, as well as from station 2 which represents the shaded canopy, so that a total of 50 research samples were collected to measure and observe the stomatal density and leaf area.

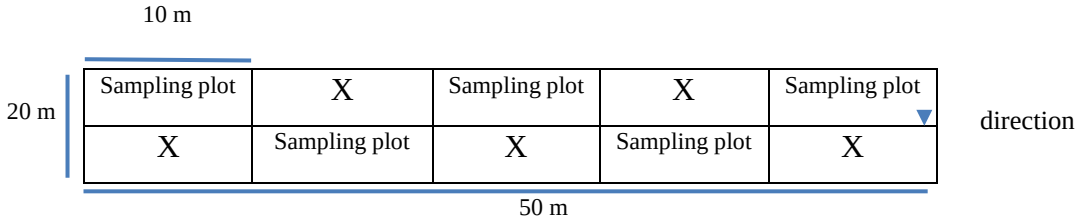

FIGURE 2. Sampling technique for each station (Alhani et al., 2015).

Research procedures

Mangrove species of *R. mucronata* Poir. located in Ecotourism were identified using the Mangrove Introduction Handbook in Indonesia (Noor, 2006) with the help of the Plant.Net application. Research samples were taken from two station locations based on the similarity of *R. mucronata* size. Thus, the independent variable is only the difference in light intensity in open, which is 4000 lux in average, and shaded canopy, (200 lux in average) locations measured using a lux meter. Station 1 is an open canopy area located on the left and right of the footbridge, while station 2 is a shaded canopy area located about 500 m from the entrance to the Mangrove Ecotourism Area. Research samples at station 2 were *R. mucronata* leaves that were covered by higher plant canopies, including by *Avicennia sp*., Fabaceae plant groups, and by higher *R. mucronata*. The samples taken were mature leaves from the 5th and 6th nodes. The similarity of leaf size and maturity is one of the control variables determined to avoid errors (Hong, 2018 & Lion, M. et al., 2020).

The stomatal sampling method used was the leaf impression method using nail polish (GTAC, 2016). The leaves of *R. mucronata* Poir. that have been taken as samples are cleaned first from dirt, dust, and mud that stick to them using tissue. The next step is to apply nail polish thinly and evenly on the abaxial side of the leaf, the location where the stomata of *R. mucronata* Poir. are located. The area of the leaf that was applied with nail polish was ± 1 cm^2, adjusting to the width of the glass object and the tape used. The nail polish spread located in the centre area of the leaf lamina was allowed to dry for approximately 10 minutes, then clear tape was applied on top. The tape was pressed to make a clearer and better print and then slowly released until the nail polish print layer stuck to the tape. After that, the tape was attached to a glass slide and labelled according

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to the station, plot, and sample number. These preparations were then observed under a microscope with 100 times magnification.

FIGURE 3. Preparation of Stomatal Molds by Leaf Impression Method research documentation)

Data analysis techniques

Data collection was done in a quantitative way by counting the number of stomata of *R. mucronata* Poir. leaves per stomatal field of view. The calculation of stomatal density was carried out using the stomatal density formula according to Kurschner and Poole (1999):

$$Stomata\ density = \frac{number\ of\ stomata}{stomata\ field\ of\ view}$$

The field of view used is 4 x slide grids measuring 0.25 mm^2 or equivalent to 1 mm^2 as an aid in observation using a microscope. Counting the number of stomata followed the counting instructions using a square area, which counts the stomata that hit the top and left sides of the square but does not count the stomata that hit the bottom and right sides of the square (**Figure 4**).

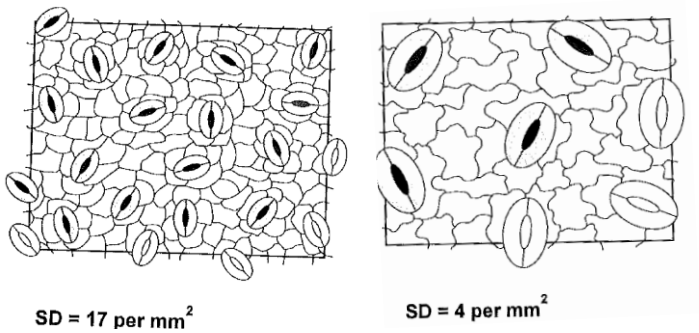

FIGURE 4. Stomatal counting technique. (SD = stomatal density) (Kurschner & Poole, 1999)

The environmental parameters as independent or comparative variables measured in this study were light intensity, which are 4500 lux on average in open area and 200 lux on average in shaded area. The leaf area of *R. mucronata* Poir. was measured using the ImageJ application. Leaf samples that had been labelled were then photographed with a background of white HVS paper along with a ruler as a comparison measurement tool. The centimetre size in the photo of the ruler is then calibrated into units for the leaf area, so that after being analysed with the ImageJ application, data on the area or area of each leaf sample is produced.

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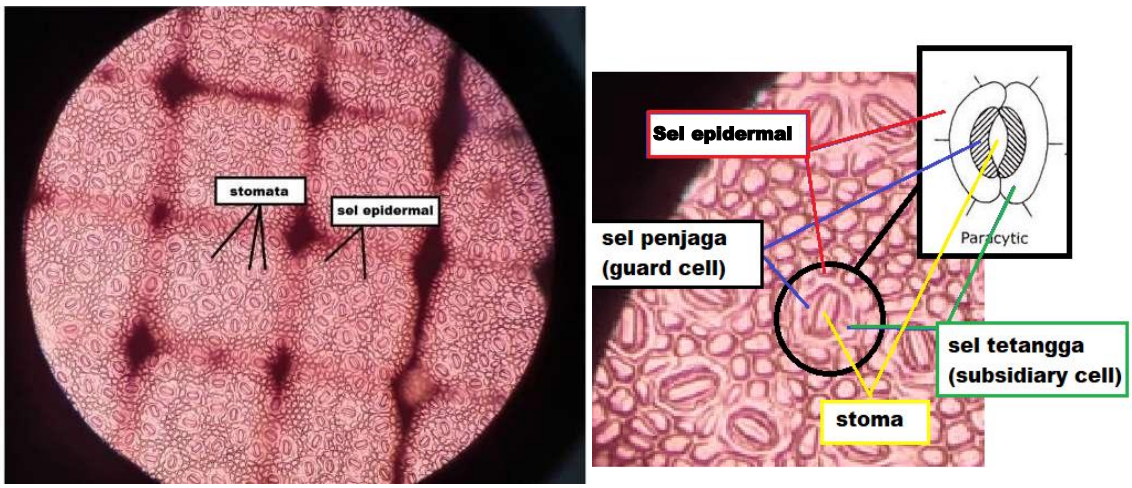
The data in this study are the average value of average leaf area and stomatal density in two locations with different canopy covers. This data was analysed through normality, homogeneity, and difference tests of two sample means t-test or independent t-test at a significance level of $\alpha = 0.05$ using the SPSS version 25 application. The results of the t-test will show the difference between the value of stomatal density and the average leaf area at open and shaded canopy location points with different light intensities in Nature Tourism Park Angke-Kapuk.

RESULTS AND DISCUSSION

Based on the results of sampling in the field, a total of 50 leaf samples were obtained consisting of 25 leaf samples from station 1 representing open canopy and 25 leaf samples from station 2 representing shaded canopy. Based on observations, it is known that the epidermal cells of *R. mucronata* Poir. are clearly more prominent than the stomata and neighboring cells, so that *R. mucronata* Poir. has cryptopore stomata type. This is similar to the results of Surya's research (2017) that the type of stomata of most mangroves is cryptopore as a morphological adaptation to reduce the rate of transpiration. According to Reef & Lovelock (2014), this stomatal morphology will not reduce the uptake of CO_2 levels needed in the photosynthesis process but suppress the transpiration rate by increasing the humidity around the stomatal pore significantly to reduce the leaf-to-air vapour pressure deficit. Judging from the type of neighbouring cells, the stomata of *R. mucronata* Poir. including parasitic type, in accordance with the results of research by Novitasari et al. (2018) and Hadi et al. (2016) which states that species of the genus *Rhizophora* have stomata with parasitic or rubiaceous types, which have two neighbouring cells with a longitudinal axis and a linear field with guard cells/closers. Observations of stomatal morphology at both stations showed similar results in terms of the shape and type of stomata, which were cryptopore and parasitic. The shape and type of stomata of *R. mucronata* Poir. observed under a microscope are presented in **Figure 5**.



(a) (b)

FIGURE 5. Observation of the shape, type, and size of stomata of *R. mucronata* Poir. (a) in one field of view (100x) (b) description of stomatal parts and epidermal cells (400x) (Research documentation)

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The results of counting the number of stomata in a field of view of 4 x 0.25 mm^2 or 1 mm^2 indicate the density of the number of stomata, while to calculate the leaf area with precision, the Image J application was used. Data on the average density of stomata and leaf area in samples in each research plot are presented in **Table 1**.

TABLE 1. The results of the two-sample t-test difference test on stomatal density

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Stomata density	Equal variances assumed	0.062	0.804	-1.592	48.00	0.11	-3.40	2.13	-7.69	0.89
	Equal variances not assumed			- 1.592	48.00	0.11	-3.40	2.13	-7.69	0.89

After the data on leaf area of *R. mucronata* Poir. was tested for normality and homogeneity, it was found that the Kolmogorov-Smirnov test results showed a significance number (sig) of 0.005 in leaf area data in open locations and 0.200 in shaded locations. Thus, the leaf area data in the open area came from a population that was not normally distributed. The results of Levene's test for equality of variances or homogeneity test showed a significance of 0.001, so it can be stated that the leaf area data is not homogeneous. Because the data is not normally distributed and not homogeneous at the significance level of $\alpha = 0.05$, then the non-parametric test of the difference between the two-sample means is carried out with the Mann-Whitney test, the results of which can be seen in **Table 2**.

TABLE 2. Mann-Whitney test results on *R. mucronata* Poir. leaf area

Ranks					**Test Statistics[a]**	
	Light Intensity	**N**	**Mean Rank**	**Sum of Ranks**		**Leaf area**
Leaf area	Open area	25	21.44	536,00	Mann-Whitney U	211.000
	Shaded area	25	29.56	739,00	Wilcoxon W	536.000
	Total	50			Z	-1.939
					Asymp. Sig. (2-tailed)	0.049
					a. Grouping Variable: Light intensity	

TABLE 3. Mean data of stomatal density and leaf area of *Rhizophora mucronata*

Plot	**Average stomatal density (/mm^2)**		**Average leaf area (cm^2)**	
	Station 1	**Station 2**	**Station 1**	**Station 2**
1	69.00	70.60	116.89	116.79
2	71.80	57.40	138.07	102.52
3	68.20	63.00	97.02	107.24
4	75.20	71.00	109.45	170.38
5	65.60	70.80	94.66	160.11
Mean	**69.96**	**66.56**	**111.22**	**131.41**
SD	**3.67**	**6.14**	**17.55**	**31.52**

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The mean stomatal density of *R. mucronata* Poir. at station 1 (open canopy) with a light intensity of 4500 lux was 69.96 stomata per mm^2, while at station 2 (shaded canopy) with a light intensity of 200 lux was 66.56 stomata per mm^2. This means that the stomatal density of *R. mucronata* Poir. in shaded and open locations do not differ significantly. The value of *R. mucronata* stomata density is not much different from the results of Ariyanto's research (2018) on the dynamics of mangrove stomata in Rembang, Central Java which states that the density of *R. mucronata* Poir. stomata are 61.24449 per mm^2. These results indicate that *R. mucronata* Poir. has stomata in the low-density category because it amounts to <300 per mm^2.

Although there is a difference in the mean value of stomatal density between the two locations with different canopies, however, the results of the difference test of the two mean samples t-test show a 2-tailed sig value of 0.118 or more than the significance level of 0.05. The calculated t value after being compared with the t table value at the 5% significance level shows the result of t count = 1.592 < t table = 2.010. These results indicate that there is an insignificant difference between stomatal density in open and shaded canopy areas (**Table 3**).

The non-significant difference of stomatal density in *R. mucronata* Poir. in open and shaded locations indicates that *R. mucronata* Poir. has various other forms of adaptation to differences in light intensity. Adaptation in the form of consistency in stomatal density or density of *R. mucronata* Poir. can also be seen in its relative, *R. mangle*. According to Farnsworth and Ellison (1996) in CABI (2021), the habitus of *R. mangle* trees growing in sunny areas has no difference in leaf anatomy (relative thickness of leaf tissue and stomatal density) compared to those growing in shaded areas.

Based on the data in **Table 3** and **4**, it can also be interpreted that the non-significant difference in mean stomatal density is inversely proportional to the difference in mean leaf area between open and shaded areas. The average data of leaf area in the open area was 111, 22 cm^2, while the average leaf area in the shaded area was 131.41 cm^2. This is not in line with the results of Sundari and Atmaja (2011) who stated that leaf area is significantly positively correlated with the number of epidermal cells and stomata; and Haryanti (2010) who explained that radiation intensity can increase leaf area. In both studies, it was suggested that an increase in the rate of photosynthesis in plants living in an open canopy would increase the amount of energy used for the division of leaf cells. However, these results do not seem to apply to all plant species. *R. mucronata* Poir. has different adaptation abilities from plants in general.

The Mann-Whitney test results show an Asymp. Sig 2-tailed value of 0.049 or less than the significance level of 0.05. These results indicate that there is a significant difference between leaf area in open and shaded canopy areas. The value of insignificant differences in stomatal density in different canopies and inversely proportional to leaf area in *R. mucronata* Poir. coupled with the results of non-parametric tests that show significant differences between leaf area in open and shaded canopies can be evidence that this species has a form of adaptation to the efficiency of photon energy capture in shaded areas by increasing leaf area in optimizing the division of epidermal cells but not increasing the number and density of stomata. This is because increasing the number of stomata will have an impact on increasing the transpiration rate (Sundari & Atmaja, 2011;

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Tihurua, E. F., et al., 2020; & Marantika et al., 2021). The low level of stomatal density of *R. mucronata* Poir. (<300 per mm^2) can be a sign of low transpiration rates in this species. It is a form of adaptation in mangroves that although living in habitats with high light intensity and temperature that can accelerate the transpiration process, mangroves are also in habitats with moderate to high salinity levels that require them to perform water storage efficiency and regulate the release of excess salt from their bodies.

However, the Mann-Whitney test value which is very close to the significance value may be a sign that the form of morphological adaptation that *R. mucronata* Poir. is not too dominant compared to its physiological adaptation, both to light intensity, transpiration, respiration, and photosynthesis processes. This is supported by the results of research by Lion et al. (2020) which stated that *R. mucronata* Poir. was able to reduce the transpiration rate to only 0.50 mmol H_2O m^{-2} s^{-1} during the day and was even more efficient in the morning, which was 0.30 mmol H_2O m^{-2} s^{-1}. This is evident in its close relative, *R. apiculata*, whose transpiration rate during the day can reach 0.92 mmol H_2O m^{-2} s^{-1} and 0.85 mmol H_2O m^{-2} s^{-1} in the morning. The physiological adaptation of *R. mucronata* Poir. is capable of efficient water storage up to 32.88 µmol CO_2 $mmol^{-1}$ H_2O.

Besides being caused by the effectiveness of water storage, *R. mucronata* Poir. tolerance to places with low light intensity is the ability to absorb carbon. According to research by Rachmawati, Setyobudiandi, and Hilmi (2014) *R. mucronata* Poir. has a higher ability to absorb carbon than other species. The stored carbon value of *R. mucronata* Poir. reached 17.60 tons/ha with a potential biomass of 34.31 tons/ha. Although the photosynthesis rate of this species is only 9.34 µmol m^{-2} s^{-1} and is classified as low (Lion et al., 2020), the amount of stored carbon is sufficient to meet the needs of glucose through the photosynthesis mechanism. This is similar to the results of Peel et al. (2017) which stated that stomatal density in the mangrove species *Rhizopora mangle* studied showed a significant negative correlation with leaf width, leaf length, leaf area, stem diameter, and tree height. Stomatal density can increase with a reduction in leaf area size to overcome the need for sufficient functional stomatal units per leaf unit to meet the CO_2 flux and light capture required in the photosynthesis process (Franks & Farquhar, 2007).

According to Giesen et al. (2006), it is known that *R. mucronata* Poir. is the only mangrove species capable of living in zone 1 which is very exposed and bordering the sea so that it is categorized as highly exposed mangrove. This species is also able to adapt to grow up to zone 3 or the central mangrove zone. This shows the high adaptability of *R. mucronata* Poir. to withstand differences in environmental conditions, including inundation conditions, substrate type and salinity, including sunlight intensity.

R. mucronata Poir. is also able to live in environments with high levels of heavy metals. This is because this species can absorb heavy metals such as Cu, Pb, and Zn at quite high levels (Hamzah & Setiawan, 2010). This condition could make *R. mucronata* Poir. adaptive and able to overcome non-ideal habitats. Even this ability has the potential to make it an effective phytoremediation agent (Purwiyanto, 2013). However, the adaptive power of this plant does not prevent it from being threatened with extinction. This is especially true due to the difficulty of propagules to grow naturally and even some are sterile (Batool, 2014). *R. mucronata* Poir. is difficult to experience growth in the wild

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or at least requires the right time and conditions. Therefore, mangrove conservation by planting regularly is important considering that this species is an essential organism in the ecosystem.

CONCLUSIONS

There are significant differences in leaf area but insignificant differences in stomatal density in *Rhizophora mucronata* Poir. which grows in open canopy and shaded canopy. This means that *R. mucronata* Poir. has a good ability to adapt to environments with low or high light intensity, which may vary according to age stage. This species has a form of adaptation to the efficiency of photon energy capture in shaded areas by increasing leaf area in optimizing the division of epidermal cells but not increasing the number and density of stomata.

AUTHOR CONTRIBUTIONS

S., B.T.W., T.W..: project conception; S., T.W., M.S..: methodology; B.T.W., T.W., M.S., A..: data analyses; B.T.W., T.W., M.S., A.: original manuscript draft; B.T.W., T.W., M.S., A.: manuscript review and editing.

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CONFLICTS OF INTEREST STATEMENT

There are no conflicts to declare.

DISCLOSURES AND ETHICS

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributor ship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects.

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