Effect of Temperature on the Physiological Response of *Enhalus acoroides* Seedlings

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Abstract

Increasing sea surface temperatures as an effect of global warming can affect the survival of marine organism, among these marine organisms is seagrass. Temperature is one factor that can determine seagrass's physiological response in maintaining its life, including in the early stages of life in seagrass seedlings. This research aims to study the effect of temperature on the physiological response of Enhalus acoroides seedlings such as growth rate, leaf tissue anatomy, and chlorophyll content. The method used was an experiment in the laboratory. The seagrass seedlings were grown in an aquarium with three sea water temperature treatments (28°C, 31°C and 35°C) for 8 weeks of maintenance. The choice of sea water temperature treatment of 28°C (A) as a control is the optimal temperature range for seagrass, the treatment temperature of 31°C (B) refers to previous study, i.e. the temperature in the area of origin of the seagrass meadow, and the treatment temperature of 35°C (C) is considered as an estimate of temperature under the scenario of. The growth rate and the average leaf length were more optimal with a high chlorophyll content found at a temperature treatment of 28°C. The highest anatomical size of leaf tissue in the upper and lower epidermis was observed at 31°C, while the most extensive mesophyll tissue was observed at 35°C. In this study, temperature significantly affected the growth rate, average leaf length, anatomical structure of mesophyll tissue, and chlorophyll content of the Enhalus acoroides seedlings.

Keywords: *Enhalus acoroides, physiological response, seeds, temperature*

Introduction

Seagrass beds are a constituent of coastal ecosystems that have an essential ecosystem service, such as habitat for marine biota, sources of primary producers, stabilizing the bottom substrate of the waters, food provision for coastal community and carbon regulation (Nugraha *et al*., 2021). The surrounding environmental conditions strongly influence the condition of sustainability of the seagrass ecosystem. One of the phenomena that can threaten the sustainability of seagrass ecosystems is global warming (Riniatsih *et al*., 2021). The phenomenon of global warming is expected to continue to increase for the next few decades (Ontoria *et al*., 2019). The Intergovernmental Panel on Climate Change predicts global temperatures will likely increase by 1.1–6.4°C in the next 90 years (Syaifullah, 2015).

Temperature is a factor that plays an essential role in the process of seagrass survival (Artika *et al*., 2020). Seagrass can grow optimally at 28–30°C (Hartati *et al*., 2012). Increasing temperature above the threshold will impact seagrass metabolism and nutrient uptake ability (Collier and Waycott, 2014). Some of the responses of *Posidonia oceanica* to rising temperatures: damage to the photosynthetic system, limitations in leaf growth, increased leaf senescence and can cause death (Guerrero-Meseguer *et al*., 2017).

Enhalus acoroides is a seagrass species that lives in tropical waters and is wide spread in the Indo-Pacific region (Artika *et al*., 2021; Sumbayak *et al.*, 2023). *E. acoroides* is a type of seagrass with good adaptability (Kilminster *et al*., 2015). This type of seagrass can produce fruit with seeds that act as a seed source for seagrasses (Ambo-rappe and Yasir 2015). Seedlings are part of the early phases of

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seagrass life, whose survival is heavily influenced by environmental factors (Artika *et al*., 2021).

The results of an experimental study conducted by Artika *et al*. (2021) stated that temperature significantly affected the growth of seagrass seedlings. In this research, a study was conducted regarding the effect of temperature on the physiological response of *Enhalus acoroides* seedlings which included growth, leaf tissue anatomy, and chlorophyll content.

Materials and Methods

Research methods and procedures

The method used in this research is an experiment in the laboratory. The research subjects were seeds from the seagrass *Enhalus acoroides* which were seeded with three temperature treatments in the aquarium. The choice of temperature treatment of 28 °C (A) as a control is the optimal temperature range for seagrass, the treatment temperature of 31 °C (B) refers to Artika *et al.* (2020), i.e. the temperature in the area of origin of the seagrass meadow, and the treatment temperature of 35 °C (C) is considered as an estimate of temperature under the scenario of climate change conditions (IPCC, 2013). The aquarium used 50x50x40 cm in size. Each aquarium contains 30 seagrass seedlings. The type of substrate used is dominated by sand. The results of the analysis of the substrate taken from Dompak Island were sand (98.6%), gravel (0.8%), and mud (0.7%). Each aquarium is filled with substrate as high as 10 cm and seawater with a volume of 45 L.

Planting Enhalus acoroides seeds

The seagrass fruit that has been taken is then opened and the seeds were collected. Usually there are 8-12 seeds in 1 fruit (Ratnawati *et al*., 2019). A total of 90 seagrass seeds that have been collected with an average weight that is relatively the same (±1.25 g) are then taken randomly to be planted on growth media into 3 aquariums.

Measurement of water quality

Measurement of the quality of sea water that has been taken and put into the aquarium includes temperature using a thermometer, degree of acidity (pH) using a pH meter, dissolved oxygen (DO) using a DO meter, and salinity using a hand refractometer. Water quality measurements were carried out every week in each aquarium for 3 repetitions.

Measurement of seagrass seedling growth

Growth of *E. acoroides* seedlings included leaf length which was measured using a 1 mm scale ruler. The growth rate of seedlings leaf length was calculated using Supriadi (2003) formula, while the length of the seagrass leaf was calculated by finding the average of 30 seagrass seedlings individuals in each aquarium. Measurements were carried out once a week for 8 weeks of experiment. Analysis of seagrass leaf growth used the Supriadi (2003) formula as follows:

$$P = \frac{Lt - L0}{\Delta t}$$

Note: P= Growth rate of leaf length (cm); Lt= Leaf length after time t (cm); L0= Leaf length at initial measurement (cm); t= Interval measurement time (days)

Observation and measurement of leaf tissue anatomy

Leaf samples in each aquarium were selected as many as 5 leaf stands using simple random sampling. The method by choosing a random sample allows members of the population to have the same opportunity to be sampled (Khairunnisa *et al*., 2022). Observations of leaf tissue anatomy were done by using a transverse incision on the leaf (Nugraha *et al*., 2022b). The sampling position was in the middle of the total length of the seagrass leaves (Soonthornkalump *et al*., 2022). Then the results of the sliced seagrass leaves were placed on a glass slide and dripped with distilled water so the leaf samples did not dry out. Observation of leaf tissue anatomy was observed using a microscope and then measured by marking on the software motic image plus 3.0 (µm) at 100x magnification with 1 repetition on the upper and lower epidermis and 10 repetitions on the mesophyll. Leaf tissue measured included the thickness of the upper epidermal layer, lower epidermal layer and mesophyll tissue (Nugraha *et al*., 2016).

Measurement of chlorophyll content

Sampling of leaves in each aquarium was selected as many as 5 leaf stands using simple random sampling. The *E. acoroides* seedlings were cut into small pieces, mashed using a mortar, and weighed as much as 50 mg using a digital scale. The sample was transferred to a test tube and dissolved in 5 ml of 90% acetone (Putera *et al*., 2021). The sample was allowed to stand for 1 min so that the chlorophyll dissolved (Nugraha *et al*., 2022b). Then it was centrifuged at 1000 rpm for 15 mins. Extraction results were measured using a spectrophotometer at wavelengths of 664 and 647 nm (Andika *et al*., 2020). Each sample extract of seagrass leaves was absorbed using 664 nm and 647 nm wavelengths. The chlorophyll content *E. acoroides* is calculated by the formula (Short and Coles, 2001):

$$\text{Chlorophyll a (mg.L}^{-1}) = 11.93(E_{664}) - 1.93(E_{647})$$
$$\text{Chlorophyll b (mg.L}^{-1}) = 20.36\ (E_{647}) - 4.68(E_{664})$$

Note: E_{664} = Absorbance wave at 664 nm; E_{647} = Absorbance wave at 647 nm

Statistical analysis

Effect of temperature on the physiological response of *E. acoroides* seedlings was analyzed using one-way ANOVA at a significant level of 5% (Andika et al., 2020). Before carrying out the ANOVA test, a normality test (Liliefors test) was first carried out to determine the normal distribution of the data. After ensuring that the data has a normal distribution, it is continued with the One way ANOVA test. One way ANOVA is used to test the average or treatment effect of an experiment using 1 of these factors with three or more groups (Siregar, 2014). If the results show a significant difference, proceed with the Least Significance Difference (LSD) test

Results and Discussion

Water quality parameters

The average temperature in this study is treatment A as control of 28.22°C, treatment B of 31.11°C, and treatment C of 34.94°C. The highest salinity was found in treatment C at 45.55 ‰, then in treatment B at 38.88 ‰ and the lowest in treatment A at 36.77‰. The highest DO value was found in treatment A of 6.94 mg.L^{-1}, then treatment B of 6.47 mg.L^{-1} and the lowest was in treatment C of 5.06 mg.L^{-1}. The highest pH value was found in treatment A of 7.29, in treatment B of 7.26, and the lowest in treatment C of 7.24 (Table 1.)

Water quality parameters play an important role and affect the survival of aquatic biota, including seagrass. The temperature quality standard's value is based on the quality standard of Regulation of Government No. 22/2021 on seagrass ecosystem, which is 28–30°C. Temperature affects physiological processes in seagrasses, such as growth rate, photosynthesis and reproduction processes (Handayani *et al*., 2016). The ability of the photosynthesis process will decrease if the water temperature is outside its range (Poedjirahajoe *et al*., 2013).

The salinity value (‰) in the three treatments exceeded the quality standard of Government Regulation. The highest salinity was found in treatment C of 45.5‰, and the lowest was in treatment A of 36.77‰. Seagrasses have different tolerances for salinity, but most seagrasses are tolerant in the salinity range of 10– 40 ‰ (Aziizah *et al*., 2016). The high salinity value is caused by the increasing temperature so that the seawater in the aquarium evaporates faster and the water volume decreases. Juniarti *et al*. (2017) stated that the high-temperature value caused the high salinity value. This was due to the evaporation process. In addition, it is suspected that there is no current because this research is still being carried out on a laboratory scale. The current influence's speed and direction determine the salinity distribution in the waters (Wardhani *et al*., 2021).

The DO value (mg.L^{-1}) in the three treatments was still within the range of quality standards according to Government Regulation, with the highest DO value found in treatment A of 6.94 mg.L^{-1}, and the lowest in treatment C of 5.06 mg.L^{-1}. This study's value of dissolved oxygen is closely related to the temperature value. Patty *et al*. (2021) stated that the higher the oxygen solubility value at low temperatures and when the temperature was high, the solubility value became lower. The dissolved oxygen content can fluctuate, one of which is related to the respiration process carried out by aquatic plants (Puspitaningrum *et al*., 2012).

The pH values for the three temperature treatments were still within the quality standard according to Government Regulation, which is 7– 8.5. For treatment A of 7.29, treatment B of 7.26, and treatment C of 7.24. Varying pH values can affect the biota in water. If the water conditions are very alkaline or very acidic, it can endanger the survival of organisms because it will interfere with metabolic and respiratory processes (Hertyastuti *et al*., 2020)

Growth of Enhalus acoroides seedlings

The results of the growth rate of leaf length for 8 weeks of maintenance have the highest value in treatment A of 0.20 cm.day^{-1}, then in treatment B of 0.19 cm.day^{-1}, and the lowest in treatment C was 0.08 cm.day^{-1} (Figure 1.). Based on the results of the one-way ANOVA test showing that there was a significant difference in the three treatments (P<0.05). A further test was carried out with the Least Significant Difference (LSD) test, so that the results of the average growth rate were obtained. There were differences in leaf length in treatments A and C as well as treatments B and C.

Growth of seedlings' leaf length until the end of the study at week 8 had the highest value in treatment A of 5.5 cm, then in treatment B of 5.34 cm, and the lowest in treatment C of 2.71 cm (Figure 2.). The results showed that the average leaf length was a significant difference in the three treatments (P<0.05). The growth rate of leaf length for eight weeks of maintenance had the highest value in treatment A of 0.20 cm, and the lowest was in treatment C of 0.08 cm.day^{-1} (Figure 1.). Nugraha *et al*. (2022a) reported that the growth rate of *E. acoroides seedlings* was 0.09 cm.day^{-1}. The research of Tasabaramo *et al.* (2015) regarding the growth rate of *E. acoroides* transplanted in monospecies, which is 0.29 cm.day^{-1}. The results of Nugraha *et al.* (2022a) the length of the seagrass leaves in the control treatment for 60 days had an average leaf length of 8.75 cm. The highest growth rate of leaf length in treatment A because the temperature treatment used was in the optimal temperature range for seagrass growth so that the seagrass seedlings could grow well. According to Hartati *et al.* (2012) the optimal temperature range for seagrass is 28–30°C. The temperature has a role in seagrass's metabolic processes, growth and survival (Artika *et al*., 2020).

Table 1. Water quality parameters

Water Quality	Value (Average ± SD)			Quality Standard (Government Regulation No. 22/2021)
	Treatment A	Treatment B	Treatment C	
Temperature (°C)	28,22±0,36	31,11±0,18	34,94±0,17	28-30
Salinity (‰)	36,77±0,66	38,88±1,36	45,55±2,65	33-34
DO (mg.L^{-1})	6,94±0,60	6,47±1,07	5,06±0,77	>5
pH	7,29 ± 0,34	7,26 ± 0,39	7,24 ± 0,39	7- 8.5

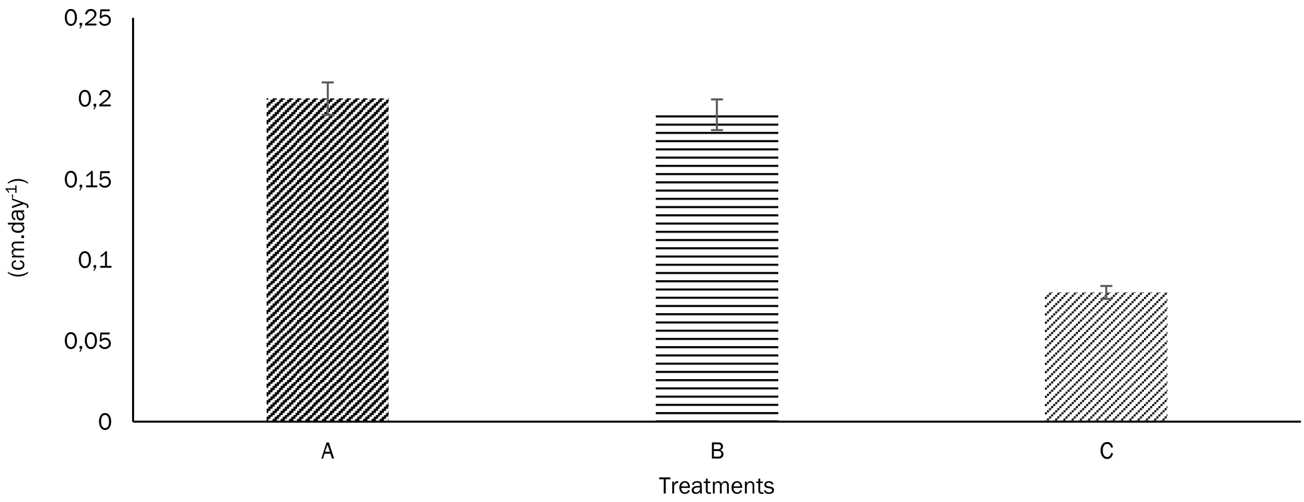

Figure 1. Growth rate of *E. acoroides* seedlings leaf length with treatment temperature (A= 28°C; B= (30 °C and C= 35°C)

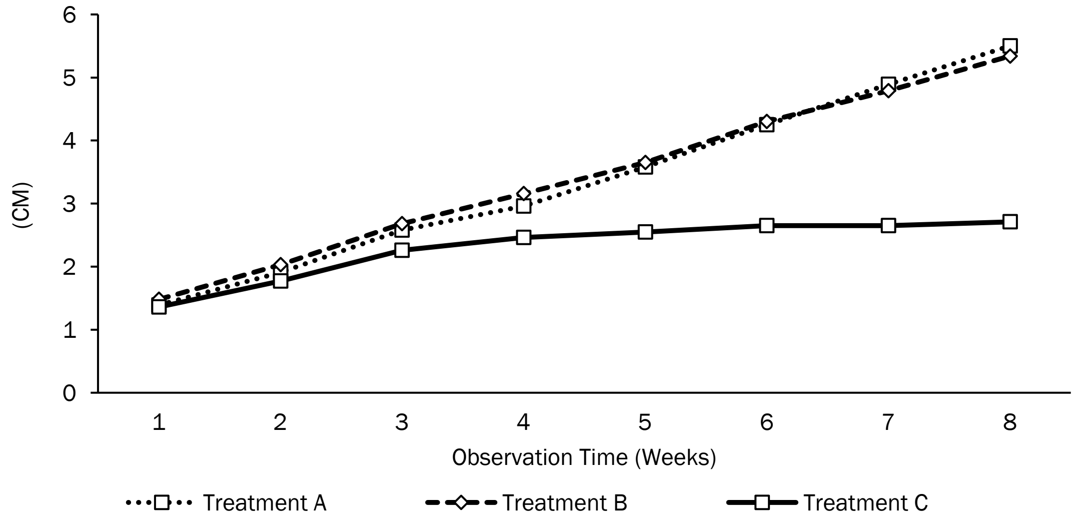

Figure 2. Growth of *E. acoroides* leaf length

The lowest growth rate in treatment C was thought to be a form of seagrass response caused by the existing temperature treatment being above the optimal temperature for seagrass so that in 8 weeks of maintenance, there was a decrease in growth rate and average leaf length of the seagrass. The response of seagrass at high temperatures can limit growth by inhibiting their photosynthetic system so that it causes decreased growth, faster leaf ageing, and in some cases, death (Guerrero-Meseguer *et al*., 2017). The existing temperature affects the physiological processes of seagrass such as growth rate, photosynthesis and reproduction processes (Handayani *et al*., 2016). Stress conditions on seagrass caused by environmental changes can occur in days to months (Andika *et al*., 2020).

The high temperature can affect the growth and morphology of the seagrass. York *et al.* (2013) study on seagrass species *Z. muelleri* in higher temperatures produce smaller leaves. According to Lee *et al.* (2007) when the temperature exceeds the threshold, it can cause a decrease in the growth in seagrass. High temperatures can cause physiological, biochemical, and molecular changes that affect growth and productivity by reducing the photosynthesis process (Song *et al*., 2014). The increase in respiration due to increased temperature over a longer period can eventually cause death in seagrass (York *et al*., 2013). Plants have defences to adapt to high-temperature stress by developing various mechanisms, including the role of Heat Shock Protein (HSP), and under high temperatures, the production of Reactive Oxygen Species (ROS) also increases, and plants must activate their antioxidant defences and protect themselves from stress hot (Purnama *et al*., 2019). Plant stress due to high temperature, salinity and others can trigger plant defences, especially through molecular mechanisms through protein formation (Wasilah *et al*., 2019).

Anatomical structure of the leaf tissue of Enhalus acoroides seedlings

The results of the measurement of the leaf tissue of *E. acoroides* seedlings, namely the measurement of the average thickness of the upper and lower epidermis tissue of leaves (Table 2.). The results of the one-way ANOVA test showed that the average results of the measurements of the upper and lower epidermis of seagrass leaves obtained that there was no significant difference (P>0.05). The highest mesophyll average size was found in treatment C of 66.27 μm, then in treatment A of 61.56 μm, and the lowest in treatment B of 59.80 μm. The results of the one-way ANOVA test showed that the average results of the measurement of the seagrass leaf mesophyll tissue was a significant difference (P<0.05).

Epidermal tissue is the outermost layer that functions as the main place for the photosynthesis process to take place, and there is a very high content of chloroplasts (Zurba, 2018). The high size of the upper and lower epidermal tissue in treatment B was thought to be a form of epidermal tissue response that was still tolerant to increased temperatures but was not far from the optimal temperature range for seagrass. The optimal temperature range for seagrass is 28–30°C (Hartati *et al*., 2012). Epidermis is the outermost layer of cells and covers the leaf surface and functions as a protective inner tissue in plants (Sari and Herkules, 2017). The low size of the upper and lower epidermis in treatment C is thought to be due to the temperature treatment being above the optimal threshold. According to Purnama *et al.* (2018) high temperature treatment can result in a decrease in the thickness of the epidermal tissue of seagrass leaves.

The results of the measurement of the highest mesophyll tissue were found in treatment C, which was also thought to be caused by temperature pressure which was above the threshold. Purnama *et al*. (2018) reported that the average value of the thickness of the seagrass mesophyll tissue of *Thalassia hemprichii* exposed to high temperatures was 49.71 μm while at normal temperatures it was 30.48 μm. The increase in the thickness of the mesophyll tissue due to high temperatures is caused by an increase in the permeability of the plasma membrane present in the leaves (Zhang *et al*., 2005 in Purnama *et al.*, 2018). According to Kaewsrikhaw and Prathep (2014), differences in environmental conditions can affect the anatomical size of leaves such as length of marginal cells, length of superficial cells, thickness of the epidermis, width and length of mesophyll cells and lacunae. This includes forms of tissue adaptation in leaves that provide physiological responses to different environmental conditions.

Chlorophyll content of Enhalus acoroides seedlings

Measurement of the chlorophyll content of the seagrass *E. acoroides* at three temperature treatments are presented in Figure 3. The highest was found in treatment A, which was 4.73 mg.L^{-1} (chlorophyll a) and 2.90 mg.L^{-1} (chlorophyll b), then treatment B was 2.34 mg.L^{-1} (chlorophyll a) and 1.41 mg.L^{-1} (chlorophyll b), and the lowest was found in treatment C of 0.76 mg.L^{-1} (chlorophyll a) and 0.68 mg.L^{-1} (chlorophyll b). Based on the results of the one-way ANOVA test on chlorophyll a and chlorophyll b, that there was a significant difference (P<0.05).

The chlorophyll content in *E. acoroides* seedlings in this study decreased with increasing temperature. The colour of the leaves at treatment C, a high temperature in this study, became brownish so that the chlorophyll content in the leaves had a low

Table 2. Measurement results of seagrass leaf tissue *Enhalus acoroides*

Leaf Anatomical Thickness (µm)	Treatments		
	28°C	30°C	35°C
Upper Epidermis	31,54±8,16	38,00±2,75	27,29±10,45
Lower Epidermis	31,49±3,62	35,72±3,57	25,15±9,88
Mesophyll	61,56±12,16	59,80±10,62	66,27±7,06

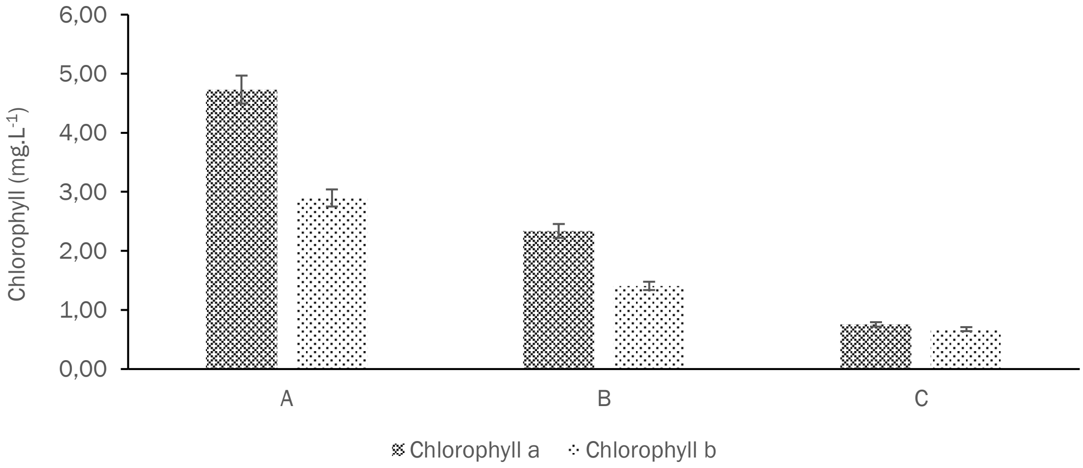

Figure 3. Chlorophyll content of *E. acoroides* seedlings

value compared to the green colour of seagrass seedlings leaves at lower temperatures. In plants, chlorophyll degradation can be caused by environmental conditions such as exposure to high temperatures (Indrasti *et al*., 2019). This causes a change in chlorophyll from green to brownish green, allowing it to turn brown due to the loss of magnesium atoms and forming its derivative compound, pheophytin (Saati *et al*., 2019).

Chlorophyll in plants is unstable, one of which is the influence of temperature so that it is easily degraded into derived molecules (Rohmat *et al.,* 2014). A decrease in the total chlorophyll content was also reported by Purnama *et al*. (2018) in seagrass *Thalassia hemprichii*, the chlorophyll content is 11.77 $mg.L^{-1}$ (normal temperature), and 5.14 $mg.L^{-1}$ (high temperature). The decrease in chlorophyll content is caused by heat stress, so chlorophyll biosynthesis is disrupted. Ramadanti *et al.* (2021) stated that the value of seagrass chlorophyll is related to the temperature value in the waters, waters with an optimal temperature value making seagrasses able to photosynthesize well.

Conclusion

The growth rate and average leaf length were more optimal, with higher chlorophyll content observed in treatment at 28°C compared to other treatments. The anatomical size of leaf tissue, the upper and lower epidermis, was highest at temperature treatment B 30°C while the highest mesophyll tissue was observed at temperature treatment 35°C. Temperature parameter in this study significantly affected the growth rate, morphometric (leaf length), the anatomical structure of the tissue in the mesophyll and the chlorophyll content of the seagrass *Enhalus acoroides* seedlings.

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