CLIMATE CHANGE FACTORS AND THE EFFECT ON *Enhalus acoroides* SEEDLINGS

Faktor Perubahan Iklim dan Pengaruhnya Terhadap Bibit *Enhalus acoroides*

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Dissertation

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DISERTASI

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Declaration

I certify that this Dissertation is compiled based on the results of research during Doctoral studies at the Faculty of Marine and Fisheries Sciences and does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university.

Makassar, May 2021

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The author is fully aware that writing a scientific paper is not easy, therefore it is possible that in the preparation of this dissertation there are weaknesses, so that I really hopes for constructive criticism and suggestions for the perfection of this dissertation.

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Author,

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Abstract

Climate change encompasses both global warming caused by human-caused greenhouse gas pollution and the subsequent large-scale weather pattern changes. Though there have been past cycles of climatic change, humans have had a genuine effect on Earth's climate system and induced environmental change since the mid-20th century. Climate change does not only affect human life but also other living things. It does not only affect areas on land, but also in waters, for example the seagrass ecosystem. Seagrasses form a seagrass bed, which is an aquatic ecosystem. Seagrasses are saltwater aquatic plants that can be found in shallow coastal waters and brackish estuarine waters. Seagrasses are flowering plants with long, grass-like stems and leaves. They have roots and rhizomes that anchor them in the sand on the seafloor, and they contain seeds and pollen. In Indonesia, as a tropical country, it has 12 types of seagrasses, one of which is *Enhalus acoroides*. *Enhalus acoroides* adults are quite resistant to environmental changes, but this does not necessarily apply to it seedlings.

By using field and laboratory experimental methods, the research wanted to prove how the influence of the factors of climate change (increased temperature and increased CO_2) on seedling *Enhalus acoroides*. Not only that, but this study also looked at the indirect impact of climate change (nutrient enrichment and heat-wave) on seedling *Enhalus acoroides*.

The results showed that climate change, through simple experiments, temperature increase and CO enrichment as well as nutrient and heat-wave enrichment had a direct and indirect impact on *Enhalus acoroides*. Directly, through increasing temperatures and heat-wave scenarios, climate change affects the metabolism of *Enhalus acoroides* seedling so that it can accelerate its growth rate. Indirectly, through CO_2 enrichment and nutrient enrichment, climate change can affect the ecosystem around *Enhalus acoroides* which can then disrupt the survival of *Enhalus acoroides*. This is related to the ability of photosynthesis and seagrass to compete with other organisms, such as epiphytes.

Ultimately, climate change could have had a greater effect on *Enhalus acoroides* if it had been exposed to it for longer. The lack of research on this topic is also the reason for the need for further and in-depth research related to climate change and its effects on *Enhalus acoroides*, especially in the seedling phase.

List of Novelties

Chapter	Type of Novelties	Novelties details
III, IV	Method/Object	First study using *Enhalus acoroides* seedling for temperature experiment
III	Method/Object	First study using *Enhalus acoroides* seedling for nutrient experiment
IV	Method/Object	First study using *Enhalus acoroides* seedling for CO_2 experiment
V	Method/Object	First study using *Enhalus acoroides* seedling on Heat-wave scenario
VI	Result	An indicator how climate change effect seagrass ecosystems.

Chapter I: General Introduction

Climate change that occurs in the world seems to be increasing lately, it is even predicted that the increase will be faster in the future. Climate change is influenced by several factors including increasing global temperature and increasing CO_2 concentration in the atmosphere. Climate change has a significant impact on life in the world, including marine life. In addition to causing direct changes to marine ecosystems, these climate change conditions also change weather conditions and season intensities, thus having an effect on nutrient enrichment in waters. As an ecosystem that lives on the coast, seagrass beds are one of the first ecosystems affected by nutrient enrichment from land. Changes in sea level, salinity, temperature, carbon dioxide, and ultraviolet radiation can affect the distribution, productivity, and population composition of seagrass because it is in an easily exposed ecosystem.

1. Seagrass

Seagrass is a symbiotic species of marine angiosperms. It has evolved three to four times from land plants to the sea. The following characteristics can be used to define seagrass species. It lives in an estuary or marine environment, nowhere else. Pollination is carried out underwater with special pollen. The seeds dispersed by biological and non-biological agents are produced underwater (Papenbrock, 2012). Seagrass species have special leaves, their epidermis is reduced, and the epidermis lacks stomata, which is the main photosynthesis organization. The rhizome or underground stem is very important in anchoring. Roots can live in an oxygen-deficient environment and rely on oxygen transport from leaves and rhizomes, but they are also important in the process of nutrient transfer (Larkum et al., 2006; Papenbrock, 2012).

Seagrass is an asexual marine plant that dominates tropical and temperate marine coastal ecosystems. It provides ecologically-related goods and services, such as food, nursery habitat, stabilizing sediment, enhancing water quality and coastline protection, and removing CO_2 are absorbed into the sediment (Nordlund et al., 2018; Orth et al., 2006a).

Seagrass is an important outpost species. It is well known that its sensitivity to changes in water quality can predict the deterioration of coastal waters. Globally, seagrass is also facing many new challenges related to climate change. These factors include the effects of temperature increase, pH decrease, and dissolved oxygen concentration (Orth and Heck, 2021).

Seagrass in the world ranges from 50 – 60 (Hemminga, 2002; Waycott, 2004), while in Indonesia there are 7 clans, namely *Enhalus, Thalassia, Halophila, Halodule, Cymodocea, Syrongidium, and Thalssodendrone* (Nontji, 1987), and consists of 12 types, namely *Halodule uninervis, H. pinifolia, Cymodocea rotundata, C. serrulata, Syringodium isoetifolium, Thalassodendron ciliatum, Enhalus acoroides, Thalassia hemprichii, Halophila ovalis, H. minor, H. decipiens, and H. spinulosa* (Hutomo, 1985). On Baranglompo Island, eight types of seagrasses were found, namely *Enhalus acoroides, Thalassia hemprichii, Halophila ovalis, Cymodocea rotundata, C. serulata, Halodule uninervis, H. pinifolia and Siringodium isoetifolium*. The distribution of seagrass is dominated by three species, namely E*. acoroides, T. hemprichii and C. rotundata*, however, single seagrass beds composed of *E. acoroides* are generally only found in areas adjacent to the coastline with a width of about 20-30 m (Supriadi, 2012).

Enhalus acoroides, is widespread, especially in fine sediments, but can also grow on medium and large rocky substrates (Hutomo, 1997). *Enhalus acoroides* usually forms pure vegetation, although this species can be found growing close to other species. Seagrass species can adapt to turbid waters due to high siltation rate (turbidity) from the mainland when the sun and the elements necessary nutrients is sufficient (Susetiono, 2004)

Seagrass profoundly affects the physical, chemical, and biological environment of coastal waters (Papenbrock, 2012). Although seagrass provides valuable ecosystem services by acting as a breeding and nursery ground for various organisms and promoting commercial fisheries, many aspects of its physiology have not been fully studied. Some studies have shown that the seagrass habitat is declining globally (Orth et al., 2006a). Ten seagrass species are at a higher risk of extinction (14% of all seagrass species), and three of them are endangered (Short et al., 2011). The loss of seagrass and the degradation of seagrass biodiversity will depend heavily on the resources and ecosystem services provided by seagrass, which will have a serious impact on marine biodiversity and population (Orth et al., 2006a; Papenbrock, 2012).

Direct effects on seagrass (for example, removal of plants during thinning) will result in immediate and quantifiable loss of seagrass. Indirect effects (such as overfishing by predators, which may reduce the food web or nutrient enrichment level) may be potentially widespread and long-term effects. Both types of losses are important, but the indirect effects may not be so obvious because the rate of decline of seagrass meadows may be slow (sometimes years or decades) and difficult to quantify. As the baseline changes over time, this slow seagrass loss may go unnoticed. Global climate change will exacerbate these effects, especially for grasslands that lack ecological resilience;

this is a major challenge for scientists who provide coastal management advice or model future trajectories (Unsworth et al., 2014).

2. Climate Change

Due to past and continuous greenhouse gas emissions caused by human activities since the Industrial Revolution (the 1900s), the global mean surface temperature (GMST) has risen by approximately ±0.8°C (1.4°F). If everyone does not take immediate action in the next ten years to reduce the rate of global warming (i.e. zero carbon dioxide emissions after 2050), scientists say that by 2050, global warming may accelerate to around 1.5°C (Taskinsoy, 2019). Due to human activity, the greenhouse gases (GHG), i.e. carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and chlorofluorocarbons (CFC) have also significantly increased in the atmosphere since pre-industrial times (Prashanth, 2020; Short and Neckles, 1999). These greenhouse gases are radioactive: they absorb thermal radiation emitted from the surface of the earth and radiate it back to the earth. Increased concentration of CO_2 in the atmosphere from 280 ppm in 1880 to almost 380 ppm in 2005 and about 30% of all CO_2 in the atmosphere resulting from burning fossil fuels is in the ocean (IPCC, 2007). Although the scale of climate change caused by the accumulation of greenhouse gases has caused great controversy among environmental scientists (Schneider, 1990, 1989), people have reached a consensus that the accelerated warming of the earth's surface has begun and will continue (Kerr, 1992; Schneider, 1994, 1990; Watson et al., 1995). The subsequent thermal expansion and melting of glaciers in the world’s oceans are expected to accelerate the rate of sea-level rise (Titus, 1990), although these predictions are controversial (Schneider, 1992). Along with the effects of global warming, the increase of carbon dioxide in the atmosphere has an impact on the photosynthesis of the earth (Amthor, 1995). Also, the accumulation of CFC and other chlorinated and brominated compounds is depleting the stratospheric ozone layer, thereby increasing the ultraviolet (UV) radiation on the earth's surface (Smith et al., 1992; Worrest, 1989).

Increasing atmospheric carbon dioxide, rising land and ocean temperatures, rising sea levels, increased ultraviolet radiation, and a series of secondary changes will change the growth conditions of terrestrial and aquatic plants. The possible impact of global climate change on natural and agricultural terrestrial plant communities has received considerable attention. On the contrary, little attention is paid to the possible impact of global climate change on aquatic plant communities, including seagrass (Watson et al., 1995).

Extensive seagrass beds provide valuable resources in shallow seas worldwide. Seagrass serves as a foraging and nursery habitat for many important commercial and recreational fish, and as food for waterfowl (Bell and Pollard, 1989; Short et al., 1989; Thayer et al., 1975). These plants regulate the dissolved oxygen in the water column, change its physical and chemical environment, and reduce the suspended sediment, chlorophyll, and nutrients in the water column (Nixon and Oviatt, 1972; Short and Short, 1984; Stevenson, 1988). The rhizome and rhizome system of seagrass restrains and stabilizes the bottom sediments (Koch and Beer, 1996; Ward et al., 1984), and the leaves of seagrass generate turbulence and improve water quality by filtering suspended matter (Short and Short, 1984).

Changes in sea level, salinity, temperature, atmospheric carbon dioxide, and ultraviolet radiation can change the distribution, productivity, and community composition of seagrass. In turn, potential changes in the distribution and structure of seagrass communities may have a profound impact on local and regional biota, coastal landforms, and biogeochemical cycles. So far, few studies have specifically focused on the potential response of seagrass to the changing global environment, but there are many documents on the relationship between the structure and function of seagrass and many environmental factors related to global climate change.

3. Temperature on Seagrass

By the end of the next century, the global average temperature is expected to rise by 1 ± 3.5°C, which will cause the temperature to rise faster than the Earth has experienced in the past 10,000 years (Watson et al., 1995). The increase in water temperature will directly affect the metabolism of seagrass and maintain a positive carbon balance (Bulthuis, 1987; Evans et al., 1986; Marsh et al., 1986; Zimmerman et al., 1989), which may lead to changes in the seasonal and geographic patterns of species richness and distribution (McMillan, 1984; Walker, 1991).

The direct effects of rising temperatures will be determined by the thermal tolerances of individual species as well as their optimal temperatures for photosynthesis, respiration, and growth. The rate of leaf respiration rises faster than the rate of photosynthesis in eelgrass, *Zostera marina*, with increasing temperature, resulting in a steady decrease in the photosynthesis-to-respiration ratio (P: R) with increasing temperature and the occurrence of a seasonal growth optimum (Marsh et al., 1986). Over an experimental temperature range, oxygen production and respiration in Mediterranean *Cymodocea nodosa* increases with rising temperature (Pérez and Romero, 1992). Temperate species such as *H. tasmanica* (Bulthuis, 1987) and *Z. marina*

(Biebl and McRoy, 1971), on the other hand, have a photosynthesis optimum at temperatures below the seasonal maximum. An increase in average annual temperature will reduce productivity and distribution for species growing in locations with temperatures above the optimum for growth or near the upper limit of thermal tolerance.

The increasing temperature may also change the distribution and abundance of seagrass by directly affecting flowering (De Cock, 1981; Durako and Moffler, 1987; McMillan, 1982) and seed germination (Garth Harrison, 1982; Phillips and Backman, 1983). Research shows that *Ruppia sp*., *Z. marina*, and *Zostera noltii*'s seed germination increased significantly (Hootsmans et al., 1987; Koch and Dawes, 1991; Phillips and Backman, 1983; Van Vierssen et al., 1984). The effect of temperature may be complicated by the interaction of salinity, as shown by Clostridium nodosum and *Zostera capricorni*, which require low temperatures for seed germination under high salinity (Caye and Meinesz, 1986; Conacher et al., 1994) Since many seagrass species have extremely strong plasticity in response to temperature differences (Phillips and Lewis, 1983), the increase in global sea temperature may not greatly change the absorption of sexual reproduction.

4. Nutrient on Seagrass

The enrichment or eutrophication of nutrients in the aquatic environment is considered to be one of the most common causes of seagrass loss (Burkholder et al., 2007; Jones et al., 2008; Short and Wyllie-Eciieverria, 1996). In some places, reduced nitrogen input has been reported to lead to the restoration of historical seagrass meadows (Greening and Janicki, 2006). Moderate leaching of bound soil nitrogen from the soil system to the aquatic system is beneficial to seagrass in nitrogen-limited coastal and estuarine environments (Short, 1987; Touchette and Burkholder, 2000); however, determining the threshold level of nitrogen concentration that negatively affects seagrass health is still a key area of research. In the past century, human activities have more than doubled the nitrogen content in the terrestrial system, greatly increasing the nitrogen load of rivers and coastal eutrophication, leading to a large reduction in seagrass (Vitousek et al., 1997).

Human activities (such as agriculture, fisheries, and seaweed cultivation) have led to land-use change and urbanization, leading to increased concentrations of nutrients and sediments in coastal waters. This increase in nutrients will exceed the capacity of the nutrient cycling system, increase the supply of organic carbon, and result in eutrophication, which is characterized by an algal bloom, epiphytic growth, hypoxic conditions in sediments, and ultimately leads to biodiversity (Khan and Mohammad, 2014; Lee et al., 2006). The effect of nutrient enrichment depends on the characteristics of a particular species, such as nutrient absorption capacity, nutrient excess levels (ranging from moderate to severe), and local natural conditions (such as ocean currents and tides). Eutrophication has been recognized as one of the most important factors affecting productivity, the dynamics of carbon change, and the growth of seagrass, and one of the main threats to coastal ecosystems. The direct effect occurs through the stability of physiological mechanisms (Bird et al., 1998; Brun et al., 2002; Burkholder et al., 1992; Invers et al., 2004; Touchette and Burkholder, 2007), which leads to increased nutrient absorption (Viana et al., 2019), nutrient imbalance (Li et al., 2019), changes in the morphological index (Mvungi and Pillay, 2019), changes in growth (Jorge Terrados et al., 1999), changes in sexual reproduction (Duarte et al., 1997)or direct ammonium toxicity (Van Katwijk et al., 1997). At the same time, the indirect effect of nutrient input is due to algal blooms (Duarte 1995; Short *et al.* 1995; Moore and Wetzel 2000; Nixon *et al.* 2001; Burkholder *et al.* 2007), which lead to light consumption or competition for nutrients (Campbell et al., 2018; Hernán et al., 2019; Jiménez-Ramos et al., 2017; Marco-Méndez et al., 2017; Tomas et al., 2015), through the ecological effects of herbivores or sediments (Jorge Terrados et al., 1999). Therefore, nutrients have a positive and negative effect on the seagrass response. Tropical systems are oligotrophic, with limited natural nutrients, so adding a small number of nutrients can quickly increase the competitiveness of primary producers.

5. CO_2 on Seagrass

Carbon dioxide is a chemical compound formed from 1 (one) carbon atom and 2 (two) oxygen atoms (CO_2) which is odorless and colorless. In seawater, carbon dioxide compounds exist in ionic and molecular forms. In the ionic form are bicarbonate ions (HCO_3^-) and carbonate (CO_3^{2-}) while in the molecular form are free carbon dioxide molecules (CO_2) and carbonic acid (H_2CO_3). These four forms of carbon dioxide are in equilibrium (Harvey, 1974).

$$\begin{matrix} CO_2 \\ \uparrow\downarrow \\ H_2CO_3 \end{matrix} \rightleftarrows HCO_3^- + H^+ \rightleftarrows CO_3^= + H^+$$

Carbon absorbed or released into the atmosphere is in the form of carbon dioxide (CO_2) gas. The ocean absorbs carbon when the partial pressure of carbon dioxide gas in the atmosphere is higher than its pressure in the seawater. On the other hand, the ocean releases carbon when the partial pressure of carbon dioxide gas in the sea water is higher than its pressure in the atmosphere.

Reversible reaction takes place quickly, so it is difficult to distinguish between carbonic acid and carbon dioxide in water. Cold sea water and turbulent weather tend to absorb carbon dioxide from the atmosphere while warmer seawater and calmer water movement tend to release carbon dioxide into the atmosphere.

Between 1980 and 1994, the ocean captured 28% to 34% of anthropogenic carbon dioxide (CO_2) emitted into the atmosphere, the subsequent increase in ocean CO_2(aq) concentration (Sabine et al. 2004; Millero 2007) resulted in a decrease of approximately 0.1 pH units of ocean surface water compared to the time before industrialization (Caldeira and Wickett, 2003). By 2100, the pH will drop by 0.3-0.5 pH units (Caldeira and Wickett, 2003). The worst-case scenario (continuous use of known fossil fuel reserves) estimates indicate that the average surface pH of the ocean can drop by up to 0.77 pH units by 2300 (Caldeira and Wickett, 2003). The extent and rate of this change may affect the physiology of marine biota, thereby affecting its competitive interactions, community composition, and key elements (such as carbon (C), nitrogen (N), and phosphorus (P)) organisms Geochemical cycle (Vezina et al., 2008).

In the case of increased CO_2(aq) concentration in the future, the acidification of seawater can offset the high pH formed by photosynthesis in such dense seagrass stands (Bjork et al. 2008), thereby enhancing the photosynthesis and productivity of seagrass. For example, in shallow coastal areas, seagrass production and branch density are higher, where volcanic carbon dioxide vents lower the pH of the water column, which is higher than elsewhere in Ischia (Hall-Spencer et al., 2008).

Seagrass can use bicarbonate ions (HCO_3^-) as an additional Ci source for photosynthesis (Beer 1989). However, it is believed that they are not as efficient as macroalgae (Beer and Koch 1996), so they are not saturated with Ci in the shallow coastal habitats where they grow in today's atmospheric equilibrium (Beer and Koch 1996; Invers et al., 2001). The general concept of Ci limitation is supported by experimental findings, that is, CO_2 enrichment increases the growth, photosynthetic rate, and leaf sugar content of *Lepidoptera maritima* (Zimmerman et al., 1995; Thom, 1996; Zimmerman et al., 1997). The enrichment of CO_2 leads to the treatment of photosynthetic Pseudomonas marinus, which significantly increases the reproductive yield, underground biomass, and vegetative reproduction of new shoots (Palacios and Zimmerman 2007); increasing the availability of carbon dioxide by acid titration also Enhance the photosynthesis of halophilic and alkalophilic bacteria (Torquemada et al., 2005) and *Poseidon oceanica* carbon budget (Invers et al., 2002). In contrast, between the four stations with a pH range of 8.2 to 7.6, no differences were found in the photosynthetic performance of the marine pseudo-simple leaf (Hall-Spencer et al., 2008), and there was no effect of increasing the above-ground CO_2 content of the productivity of *Z. marina* (Palacios and Zimmerman 2007).

6. Heat Wave on Seagrass

As the concentration of carbon dioxide in the atmosphere increases, the average temperature of the atmosphere also increases. Related to climate change is the increase in the frequency, intensity, and duration of heatwaves on the land (Coumou and Rahmstorf, 2012). Climate change has also caused heatwaves inside the ocean, called Marine Heat Wave (MHW), which are defined as "discrete long-term abnormal warm water events" (Frölicher and Laufkötter, 2018).

Ocean heat waves cause the loss of basic seagrass species, which form organic-rich sediments under the canopy (Borum et al., 2016; Marbà and Duarte, 2009; Nowicki et al., 2017; Wernberg et al., 2012). Under climate change, the loss of seagrass and the subsequent erosion and remineralization of its sediment carbon storage may continue or intensify (Waycott et al., 2009), especially in areas where seagrass lives near its thermal tolerance limit (Walker et al., 2004).

In 2010/2011, a heat wave event occurred in Shark Bay (Shark Bay is one of the largest seagrass beds in the world (Walker et al., 1988) and is also in Indonesia), resulting in a decrease in the area of seagrass above sea level and underground biomass (Nowicki et al., 2017; Thomson et al., 2015). In the past 2 months, ocean heat waves have increased the water temperature by 2-4°C above the long-term average (Wernberg et al., 2013). These events are related to the very strong La Niña phenomenon during the summer, leading to increased drainage of warm tropical waters to the coast of Western Australia. With the increase in temperature in the southeastern part of the Indian Ocean and the continental shelf of Western Australia (Pearce and Feng, 2007), the Shark Bay seagrass bed is located at the northern edge of its geographic distribution, so they are facing the danger of further ocean heating and extreme temperature extremes. This trend has the potential to accelerate the loss of one of the largest remaining seagrass ecosystems and lead to massive carbon dioxide emissions (Arias-Ortiz et al., 2018).

7. Aims and Scope of The Thesis

As one of the countries with the longest coastline in the world, and with a wealth of biodiversity, Indonesia still pays less attention to the seagrass ecosystem and also to the issue of climate change. The lack of research on these two topics in Indonesia shows a lack of concern for the environment, especially regarding the seagrass ecosystem and the issue of climate change.

The purpose of this thesis is to discuss how several factors of climate change have an impact on the seagrass ecosystem. Especially in tropical seagrass species, namely *Enhalus acoroides*, and more specifically in the initial phase of seagrass growth, namely in the seedling phase.

The scope of this thesis focuses on the biggest factors in climate change, namely the increase in CO_2 and temperature. Apart from looking at the direct effect, this thesis also looks at what if the indirect factors of climate change play a role. In this case, nutrient enrichment is one of the indirect factors in the impact of climate change on waters.

Chapter II: Decreasing pH on seagrass epiphyte community

1. Introduction

Global Climate Change which is happening today is a serious conversation among researchers. Several studies have been conducted to prove the occurrence of global climate change. Increasing CO_2 and temperature concentrations are two direct impacts of climate change.

Increased CO_2 is considered to be the most critical problem due to its widespread global effect and cannot be recovered ecologically (*Clim. Stab. Targets Emiss. Conc. Impacts over Decad. to Millenn.*, 2011). For approximately 250 years, atmospheric CO_2 concentrations increased by 40%, from the pre-industrial era of 280 ppmv (part per million volume) to 384 ppmv in 2007 (Solomon, et al., 2007). This increase occurs because of anthropogenic activities such as industrial activities and the number of deforestation (Doney and Schimel, 2007) but this has little impact on land due to absorption by the sea (Sabine & Feely, 2007); (Sabine, et al., 2004). The absorption of this CO_2 causes a decrease in the pH value at sea, known as ocean acidification (Doney, et al., 2009).

Since the pre-industrial era, the average sea pH has been decreased to 0.1 units from 8.21 to 8.10 (Society, 2005) and this pollution situation will decrease pH from 0.3 to 0.5 units (Caldeira & Wickett, 2003) if the concentration of CO_2 in the atmosphere reaches 1000 ppmv (IPCC, 2014)

In normal condition, seawater containing 100 ppm CO_2. CO_2 in seawater changed to HCO_3^- and CO_3^{2-} which used by seawater plant as carbon source for photosynthesis (Zottoli, 1978). Some reviews indicate the impact of CO_2 and climate change on marine ecosystems (Hoegh-Guldberg & Bruno, 2010) one of which is epiphytes on seagrass.

Seagrass ecosystems has an important role in the cycle of carbon dioxide in the ocean. In the seagrass ecosystems, there is plants attached to the leaves of seagrass called epiphyte (Short and Coles, 2006).

As an important component in the ecosystem, seagrass epiphyte also directly and indirectly affected by the increase in CO_2 concentration. An epiphyte is an organism that grows on the surface of a plant and derives its moisture and nutrients from the air, rain, water (in marine environments) or from debris accumulating around it. Epiphytes in marine systems are species of algae, bacteria, fungi, sponges, bryozoans, ascidians, protozoa, crustaceans, mollusks and any other sessile organism that grows on the surface of a plant, typically seagrasses or algae (Larkum et al., 2006). This study shown the interaction between the ocean acidification on the community structure of seagrass epiphyte. Campbell and Fourqurean (2014) observed that increasing CO_2 concentration has an impact on the increased growth of filamentous algae epiphyte communities but decreased growth of epiphyte community of coralline algae in *Thalassia testudinum*. In other research of Burnell, et al (2014) shown there is a negative impact to filamentous algae growth with increasing CO_2.

2. Material and Method

2.1. Study Site

This study was conducted on seagrass bed in Southeast Barrang Lompo island, Makassar, South Sulawesi, Indonesia (05^{o}03'06" LS and 119^{o}19'52.6" BT) at 3 m depth. The seagrass bed dominated by mono-species *Enhalus acoroides* with some volcano sand made by shrimp around. It is located near coral reef and the sediment were composed by sand and coral rubble.

2.2. Experimental Design

The short-term field experimental design manipulated by CO_2 to decrease the pH from January 2015 to February 2015 with 3 factorial designs. These factorial designs consisted of three level treatment which is chamber with CO_2 enrichment, chamber without CO_2 enrichment and open plots without chamber and CO_2 enrichment. There are 12 open top chambers made by transparent acrylic and 6 bamboos open plots arranged in line design with 6 replication each treatment. Circular chamber (0.24 m^2) were spaced 0,5 m-intervals between chambers.

2.3. CO_2 Enrichment

CO_2 enrichment was organized to decreasing pH (see Campbell & Fourqurean, 2011 for detailed description). The CO_2 enrichment was conducted by adding CO_2 with concentration around 800 – 1000 ppm. This CO_2 was injected directly using pump and hose to the acrylic chamber. The level of CO_2 concentration was controlled and set to approaching the IPCC forecasts for 2100, i.e.; pH decreasing of 0.3 to 0.5 unit with 100% bubbling of 800 to 1000 ppm CO_2 (Caldeira & Wickett, 2003). The chambers were cleaned of epiphytes every once a week and to monitor the quality of the water, water quality measurement were taken in all chambers and open plots during 12:00 – 15:00 every day. Water quality measured were pH, temperature, salinity, dissolve oxygen, conductivity and photosynthetically active radiation with collected 500 mL water sample in every treatment. Salinity, dissolve oxygen and temperature was measured with DO Meter OXY type 1970i, whereas pH was measured with Multi-parameter WTW Multi type 3400i and photosynthetically active radiation (PAR) was measured with Light meter LI-COR type LI-250A.

2.4. Epiphyte Sampling

Epiphyte sampling was conducted after the field experiment. Seagrass that harvested then scanned using scanner with high resolution (1200 dpi). The scanned result divided by 4 level, i.e., bottom section (0 – 30 cm), mid-section (30 – 60 cm), top section (60 – 90 cm) and tip section (> 90 cm), give a 1 cm^2 grid and then saved as JPG. Identifying and counting the number of epiphyte community was done by seeing the picture on each 1 cm^2. This step is done for all parts of the seagrass leaves in every replication treatment.

2.5. Statistical Analyses

Water quality parameter were analyzed using Microsoft Excel to find the trend and compare it between treatment. Measure of epiphyte abundance was analyzed using SPSS 15, one way ANOVA. When significance was detected, then a post-hoc follow-up test is performed using the Tukey test, where the significance level was to 0,05.

3. Result

Table 2.1. Experimental water quality parameters. Values are given as means (min-max).

Parameters	CO_2	Non-CO_2	Control
pH	7.71 (5.8 – 8.8)	8.26 (6.8 – 8.8)	8.24 (6.9 – 8.8)
Temperature (°C)	29.27 (26.67 – 31.77)	29.34 (26.63 – 32.08)	29.32 (26.47 – 32.22)
Salinity (‰)	30.9 (20.77 – 33.08)	30.9 (19.68 – 33.02)	31.1 (20.82 – 33.05)
Dissolve Oxygen (mg/L)	6.66 (4.37 – 7.75)	6.71 (4.56 – 7.83)	6.67 (4.42 – 7.95)
PAR (µmol/m^2/s)	579.50 (66.14 – 1451.36)	623.06 (58.26 – 1481.53)	507.41 (41.00 – 1518.31)

pH value in CO_2 treatment is 5.8 – 8.8, whereas in the non-CO_2 treatment is 6.8 – 8.8 and the control treatment is 6.9 – 8.8. There are no different value between non-

CO_2 treatment and the control treatment. The average value of pH on CO_2 treatment is 7.71, on non-CO_2 treatment is 8.26 and control treatment is 8.24.

The value of the temperature in each treatment indicated the same value. From all the treatments show average temperatures range between 27°C – 32°C. Salinity values at each treatment ranges between 30-35 ‰.

The results of the dissolved oxygen measurement on any treatment are 6.66 mg/l at the CO_2 treatment, 6.71 mg/l of each CO_2 treatment, and 4.75 mg/l on the control treatment.

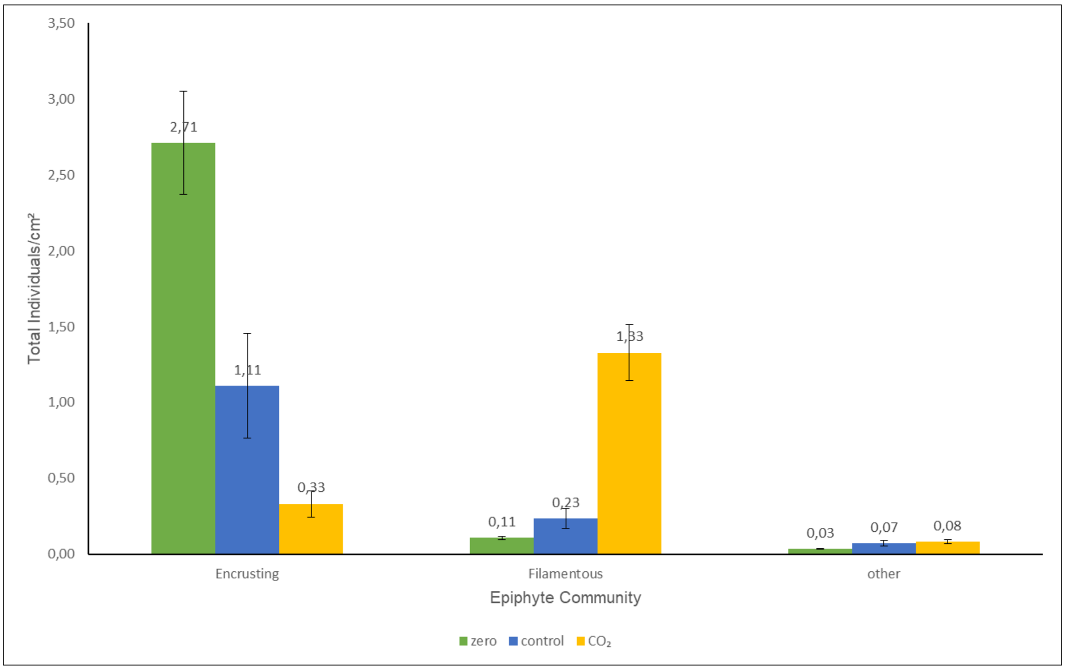

Figure 2.1. Epiphyte community found are Encrusting Algae, Foraminifera, Filamentous algae, mollusks, Spirorbis, larvae and Bryozoans

4. Discussion

Epiphytes are important primary producers in the seagrass ecosystem and make a significant contribution to the food chain. The contribution of epiphytes can reach more than 50% in the food chain in seagrass beds (Borowitzka and Lethbridge, 1989). Epiphytes are also an important food source for herbivores associated with seagrass beds (Fry, 1984).

This study shows the differences between the treatment of CO_2, non-CO_2 and the control treatment to the community of epiphytes. On these three treatments, encrusting algae look to dominate the treatment of non-CO_2 and CO_2 treatment compared with the control. A clear distinction was also presented to the community of epiphytic filamentous algae in the third treatment, in which the community is more dominant epiphytes on CO_2 treatment compared to non-CO_2 and control. Burnell et al. (2014) also suggested the same thing, that the dominant filamentous algae in the treatment of adding CO_2 and high light on seagrass kind *Amphibolis antarctica*. In addition, Campbell and Fourqurean (2014) found a decrease in the growth of algae in seagrass epiphytes Encrusting *Thalassia testudinum* kind due to the decrease in seawater carbonate and enhance the growth of epiphytic types of filamentous algae.

The increase in CO_2 is indirectly detrimental to seagrass as it can increase photosynthesis leading to abundance of algae (Short & Neckless, 1999). Increasing epiphytic cover from increased CO_2 has the potential to accelerate the destruction of the world's seagrass which has been lost to nearly a third over the past 130 years (Waycott, 2009).

The addition of CO_2 to the CO_2 treatment were carried out for ± 45 days lowers the pH value of 0.3-0.5 affecting communities seagrass epiphytes on the leaves with the growth of filamentous algae, while the non-CO_2 treatment and control showed no difference. The same is expressed by Short and Neckles (1999) who found that short-term CO_2 enrichment will increase photosynthesis, growth, total biomass, the ratio of root / shoot ratio of carbon and nitrogen from green plants in the sea.

While encrusting algae growth rate decreased due to lower pH value 0.3-0.5 CO_2 treatment unit in encrusting algae is very sensitive due to the increase of CO_2 that affects the decline CO_3^{2-} used in the process of calcification. Similar research results obtained by the Hall-Spencer et al. (2008); Martin et al. (2008); and Porzio et al. (2011) who argued that organisms have a skeleton that will be affected by an increase in CO_2 due to a decrease of carbonate in seawater.