Jurnal Kelautan Tropis Maret 2024 Vol. 27(1):81-92 P-ISSN : 1410-8852 E-ISSN : 2528-3111

Effect of Liquid Fertilizer on Seedling *Enhalus acoroides* Seeds (Linnaeus f.) Royle 1839 (Fam: Hydrocharitaceae)

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Abstract

The decrease in seagrass coverage and ecosystem harm to seagrass meadows is an annual occurrence resulting from both natural and human activities. Seagrass seeding has been deemed an effective restoration method, but its application is restricted by suboptimal environmental conditions and constraints associated with directly planting seagrass seeds in their natural habitat. The influence of environmental parameters, particularly nutrients, significantly affect on seagrass seed survival. NPK liquid fertilizer is commonly used in aquatic plants and is readily accessible in the market. It serves as a crucial source of macronutrients for seagrass. This study investigates the impact of different concentrations of NPK liquid fertilizer on the survival rate, growth, and chlorophyll, a content of *E. acoroides* seedlings. The study took place between March and May 2023 at the marine biology laboratory of the marine science study program at Diponegoro University's Faculty of Fisheries and Marine Science. According to the Manova statistical test, the NPK liquid fertilizer had an impact on the growth rate, biomass, and chlorophyll-a. The seagrass seeding container of *E. acoroides* treated with a liquid fertilizer concentration of 4.5 ml/100l exhibited the highest average growth rate of 0.29 cm/day and a chlorophyll-a value of 12.395 mg/g, with a survival rate of 100%. Causal connections between statements ensure logical construction. In contrast, seedlings without liquid fertilizer treatment showed the lowest growth rate of 0.19 cm/day and chlorophyll-a values of 5.169 mg/g, with a survival rate of 85.19%. Technical term abbreviations such as 'cm/day' and 'mg/g' are explained when first used to ensure clarity. Based on these findings, using liquid fertilizer with a concentration of 4.5 ml/l exhibits potential for restoring seagrass ecosystems.

Keyword: *Seagrass Seedling, Growth Rate, Chlorophyll-a*

INTRODUCTION

Seagrasses are flowering marine plants that serve as a source of primary productivity, foraging and spawning habitat (Kawaroe *et al*., 2016); sediment trap, nutrients trap and participate in nutrient cycling (Weitzman *et al*., 2015); dampen waves and stabilize sediments (Phair *et al*., 2020). Seagrasses also contribute to climate change mitigation by reducing CO_2 concentrations in seawater (Fourqurean *et al*., 2012). However, seagrass areas are declining due to various natural and anthropogenic factors (Waycott *et al*., 2009). Duarte *et al*. (2010) conducted monitoring in their study with the results of an estimated global seagrass area decline of 2-5% per year. The global loss of seagrasses can severely impact coastal ecosystems, including reduced biodiversity, increased coastal erosion, and degraded water quality (Amone-mabuto *et al*. 2022). In an effort to restore and conserve seagrass ecosystems, restoration approaches have become a major focus.

Seagrass transplantation, often implemented to address seagrass decline, entails the transfer of seagrass beds from donor locations to newly designated areas for planting. However, this method exhibits certain limitations, including low survival rates and reduced seagrass beds in the donor region (Zhang *et al*., 2020). Although transplantation is generally considered feasible (Wismar *et al*., 2023), it can be expensive, laborious, and time-consuming, particularly for large seagrasses like *E. acoroides* (Irawan, 2018). Additionally, this approach can potentially disrupt indigenous seagrass meadows of *E. acoroides*, reducing genetic diversity. The success rate of seagrass restoration trials is worrying, with only 37% surviving three years after planting (van Katwijk *et al*., 2009). This raises concerns about the possibility of even lower success rates. To ensure successful restoration on a large scale, it is essential to develop new methods and gain more knowledge to overcome the ecological barriers. Therefore, it is crucial to comprehend the suitable techniques for expanding restoration efforts while factoring

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Diterima/Received : 30-11-2023, Disetujui/Accepted : 03-01-2024
DOI: https://doi.org/10.14710/jkt.v27i1.21066

in each approach's associated costs and advantages, especially in light of the factors that impede restoration success. Seagrass seed implementation has recently been subject to research aiming to generate new seedlings (Ambo-Rappe., 2015; Artika *et al*., 2021). Using seagrass seeds for restoration presents several benefits. These include reduced damage during seed harvesting and less degradation of the donor site when compared to harvesting adult plants. Additionally, there is the potential for greater genetic diversity (Yue et al., 2019).

However, direct seeding of *E. acoroides* seeds in the wild has a high failure rate due to unsuitable environmental conditions and seed predation (Orth *et al*., 2020). To overcome this problem, *E. acoroides* seeds can be collected from the wild and grown under protected conditions (Ambo-Rappe *et al*., 2019). The availability of nutrients in the seagrass environment can impede growth; therefore the efficiency of nutrient cycling is crucial for seagrass productivity (Vieira *et al*., 2022). Seagrasses acquire nutrients through two tissues, roots and leaves. Nutrient absorption occurs in seagrasses with leaves drawing in nutrients from the water column and roots absorbing nutrients from sediment. It remains possible that nutrients transported by the roots can reach the leaves (Nielsen *et al*., 2006). Nitrate and phosphate act as essential nutrients for plant growth and metabolism, in addition to serving as indicators of the quality and fertility of a body of water (Fachrul, 2005). Research on seeding seagrass seedlings with nutrients, specifically ammonium nitrate and potassium phosphate, was conducted by Artika *et al.* (2020). While these nutrients can affect seagrass growth, they come at a relatively high cost. On the other hand, Wismar *et al.* (2023) found that the addition of decastar fertilizer did not significantly impact the growth of early seagrass seedlings, likely due to its slow dissolution in water.

NPK fertilizers provide the essential nutrients nitrogen (N), phosphorus (P), and potassium (K) in both single and compound forms. The addition of liquid NPK fertilizer simultaneously supplies these elements, each with a distinct function (Dewi *et al*., 2016). Alongside these elements, NPK fertilizers offer additional nutrients, including Sulfur. Plants use nitrogen to promote overall and vegetative development, including leaf growth. Phosphorus is utilized for energy transport from metabolic processes and to induce flowering and fruiting. Potassium is involved in the transport of assimilates, minerals, enzymes, and water, as well as the process of photosynthesis. Meanwhile, sulfur contributes to amino acid formation and shoot development (Shinta *et al.*, 2014).

Therefore, to produce healthy seagrass seedlings for ecosystem restoration using seeds in the future, it is necessary to study the growth response of seagrass seedlings to nutrient enrichment with commercial NPK liquid fertilizer during the early phase of life. This laboratory study focused on *E. acoroides* seedlings to maintain controlled environmental conditions. This study examined the effects of three different concentrations of NPK liquid fertilizer (1.5ml/l, 3ml/l, and 4.5ml/l) on the growth, survival rate, and chlorophyll-a of *E. acoroides* seeds. The results of this study can provide valuable information for seagrass seedling nursery technology by identifying the optimal concentration of liquid fertilizer for seedling *E. acoroides* seeds.

MATERIALS AND METHODS

This research was conducted in the marine biology laboratory of Marine Science Study Program, Faculty of Fisheries and Marine Science, Diponegoro University. Seagrass fruits of *E. acoroides* species and sediments were obtained from Karimunjawa Waters, Jepara Regency (Figure 1). The research location for seed and sediment collection was determined using field observation and purposive sampling methods. Mature *E. acoroides* Fruits are identified by the firmness of the fruit stalk and the shortening of the fruit hair, which loses its stiffness. According to Nugraha et al. (2021), the fruit of *E. acoroides* is harvested by picking it from the stalk, which is located approximately 5 cm away from the fruit. The harvested fruit is then placed in a container filled with seawater and transported to the laboratory. Sediments are taken from the location where seagrass *E. acoroides* grows, where seagrass *E. acoroides* is often found in muddy sand sediments (Chamidy *et al*., 2020). Sediments that have been taken are put into ziplock plastic. The samples were stored in a coolbox filled with ice cubes and then brought to the laboratory.

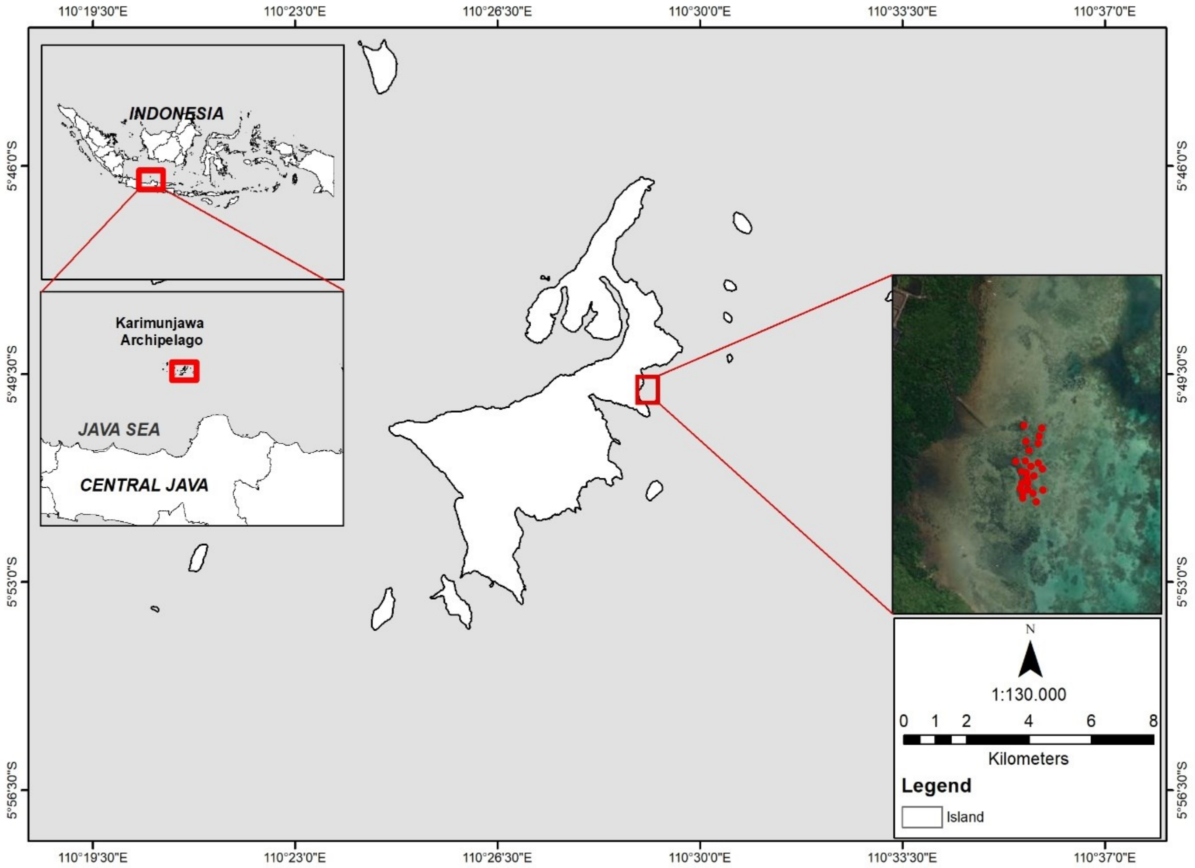

Figure 1. Map of Seagrass seeds and sediment collecting from Karimunjawa Island

Seedling Process in Laboratory

E. acoroides fruits are placed in an aerated seedling container, and after waiting for the fruit to open, seeds are carefully extracted without damaging the seed coat. Seeds of comparable size are then selected and sown in the seedling containers that have been prepared (Li *et al*., 2021). Seagrass seedlings were cared for in containers filled with seawater and equipped with aerators for circulation and LED lights, following a 12:12 hour light-dark photoperiod (Artika *et al*., 2020). Each treatment was repeated thrice to ensure the accuracy of the experiment. The seedlings were subjected to four different NPK liquid fertilizer concentrations, namely 1.5ml/100l, 3ml/100l, 4.5ml/100l, and a control group without any fertilizer.

Sediments collected from the field are placed in polybags. Seagrass seeds are then added to the polybags with the sediments before being placed in seedling containers. The seedling containers are glass cubes measuring 30 x 30 x 30. Each contains 9 seagrass seeds (Figure 2). For measuring growth, markers are placed on each polybag containing seeds. Seagrass seedling growth was measured three times a week, calculating the number of leaves, seagrass growth, survival rate, and water quality (Ambo-Rappe *et al*., 2015).

The experimental design used in this study was a completely randomized design (CRD) (figure 3) with dependent variables (responses) observed including seagrass leaf growth, seagrass survival rate, and chlorophyll a (Rappe et al., 2019). In this study, the independent variable was the treatment of different fertilizer concentrations. Each treatment was replicated three times, resulting in a total of 12 seedling containers.

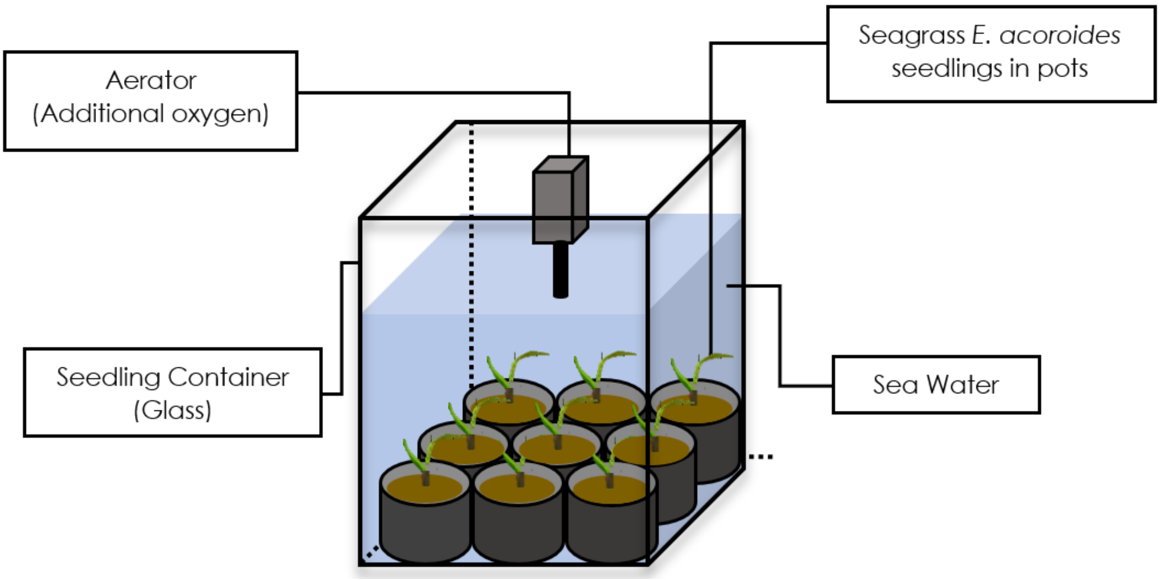

Figure 2. Seedling process of *E. acoroides* seeds in containers

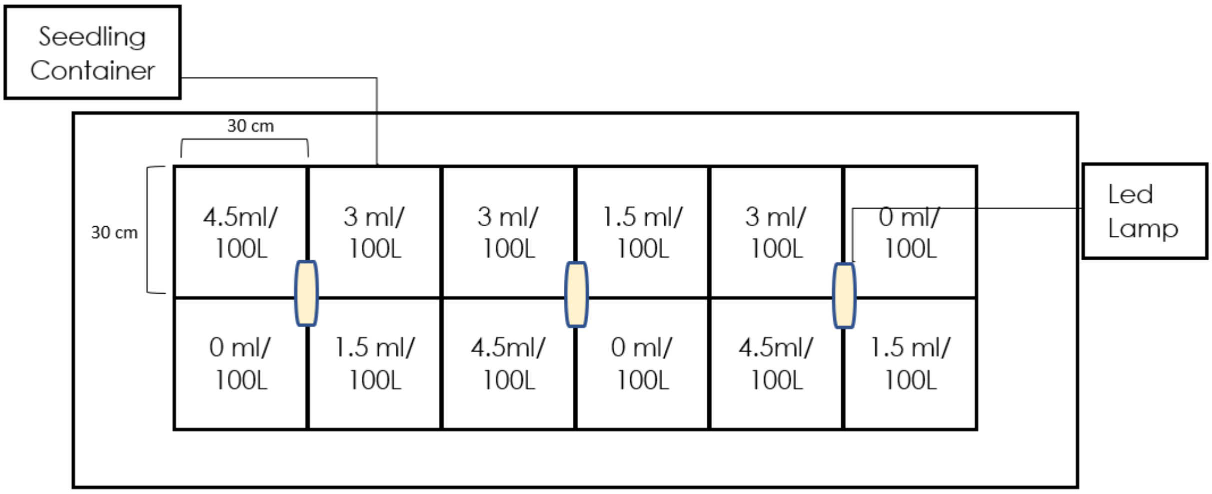

Figure 3. Completely randomized design of seedling containers

The experiment lasted for 8 weeks, from April 23 to June 10, 2023, until the seagrass seeds had developed complete root, rhizome, and leaf structures, with leaf lengths ranging from 12 to 16 cm, allowing for transplantation in the wild. Temperature, salinity, and pH of the water in the seedling container were observed three times a week (Artika *et al*., 2020). The water was changed every week due to the lack of recirculation in the observation seedling container.. Water nitrate and phosphate levels were measured twice weekly at the start and end of each week using the Hanna marine nitrate phosphate checker.

Survival Rate, Growth Rate, Biomass, and Chlorophyll-a

Observations were made on each individual seagrass plant in their designated growth container from the first to the ninth stand in sequential order. Weekly measurements of seagrass leaf growth were obtained by observing the plants three times a week. The viability of seedlings grown in a cultivation vessel was assessed using the seedling cultivation unit, and the survival rate for the seagrass seedlings already planted was calculated. Water conditions such as temperature, salinity, and pH were also closely monitored. Biomass and chlorophyll-a content measurements were taken from *E. acoroides* seagrass seeds at the conclusion of the observation period to analyze plant response to diverse liquid fertilizer concentrations. To replicate the experiment, one leaf strand from three separate stands in each container underwent chlorophyll-a sampling (Pratama and Laily, 2015). Biomass testing of seagrass seeds, three stands per container were sampled.

Data was collected weekly on the survival rate of transplanted E. acoroides seagrasses by recording the number of units and stands that remained alive. The length of seagrass *E. acoroides* leaves was measured using a caliper or folding ruler with a scale of 1 mm. The value of leaf length is used to measure leaf growth with units in cm/day. The formula for seagrass leaf growth is as follows (Short and Duarte, 2001)

$$\text{Kt} = \frac{at-bt}{T}\text{......................(1)}$$

The seagrass leaf growth value (Kt) is calculated by subtracting the total length of leaves at the beginning of observation (bt) from the total length of leaves on day t (at), and then dividing the result by the observation interval time (T). For measuring seagrass biomass, samples were collected and sorted by tissue: roots, rhizomes and leaves, then finely chopped and weighed in the wet state. To remove the samples' water content, each seagrass stand was cleaned, dried, and weighed in the laboratory using an oven set to a fixed temperature of 60°C for 24 hours. Biomass per seagrass stand can be determined by weighing the dry weight and recording the results (Supriadi *et al*., 2012). Our study used These values to identify differences among seagrass seedling treatments. We measured the biomass by obtaining the dry weight of each seagrass stand and calculating it with the formula referred to in Burnel *et al*. (2014), as follows:

Biomass (gdw.plant-1) = Total dry weight (gdw)/ Seagrass Stand......................(2)

For measuring chlorophyll-a in seagrass leaves, the sample preparation procedure involved crushing the seagrass leaves with a mortar and pestle until smooth. Then, an analytical balance was used to weigh the crushed sample, which weighed about 3 g. The weighed sample was then dissolved in 5 ml of 90% acetone in a test tube. After adding the acetone solvent, the test tube was closed and centrifuged at 1000 rpm for 15 minutes (Wagey, 2013). The centrifuged sample results were transferred carefully to a vial bottle and stored in a cool box, shielded from light. The seagrass leaf sample's chlorophyll-a content was measured at the Environmental Engineering Laboratory in the Joint Lecture Building of the Faculty of Engineering at Diponegoro University. The chlorophyll-a content is measured by transferring sample extraction results from a test tube to a UV-vis spectrophotometer cuvette, and then taking absorbance samples using wavelengths of 664 nm and 647 nm (Granger and Izumi, 2002). The chlorophyll content in seagrass leaves is determined using a UV-Vis spectrophotometer, and its value is obtained based on the calculation formula according to Granger and Izumi (2002)

Clo a = 11,93(Abs 664) – 1,93(Abs 647) ....(3)

Multivariate analysis of variance (MANOVA) is a statistical procedure that simultaneously assesses the impact of independent variables with categorical scales on multiple dependent variables with quantitative data scales. MANOVA tests whether significant differences exist between two levels of one variable in several concurrent variables (Zamani *et al*., 2018). Manova will analyze data to determine if liquid fertilizer concentration (independent variable) affects seagrass survival, growth, biomass, and chlorophyll-a (dependent variable). The study's hypothesis is H0, indicating no liquid fertilizer influence on seagrass growth, survival rate, and chlorophyll-a. H1: The liquid fertilizer influences the growth, survival rate, biomass, and chlorophyll-a seagrass levels.

RESULTS AND DISCUSSION

The statistical analysis using Manova indicates that liquid fertilizer does not significantly impact the survival rate value (p> 0.05). Nevertheless, the average survival rate of *E. acoroides* across all liquid fertilizer treatments was notably high, ranging from 85.19% to 100% (refer to Figure 4). A recent study by Sinclair *et al*. (2021) reported on a large-scale seed-based restoration of Posidonia australis in Cockbund Sound, Western Australia, where more than 200,000 seeds were manually applied to the seabed. After one year, the seedling survival rate was reported to be approximately 10%. Similarly, Ambo-Rappe (2022) conducted a study on seagrass seed survival rate by planting seeds directly in the wild, which was about 64%. It was observed that seagrass seeds tend to germinate rapidly under suitable conditions in the wild. It is commonly held that laboratory nurseries offer more controlled environmental conditions. A significant constraint of seedling establishment programs is the seedlings' low survival rate, which is attributed to unsuitable environmental conditions (Ambo-Rappe *et al*., 2019). *E. acoroides* seedlings experience high failure rates in their natural habitat due to environmental conditions after being released (Ort *et al*., 2014).

Seagrass seedling observation under liquid fertilizer concentration treatment of 4.5 ml/L yielded an average survival rate of 100% throughout the observation period. However, seagrass seeds exposed to a liquid fertilizer concentration treatment of 3 ml/100l recorded a decrease in survival rate during weeks 4, 5, and 6 with an average survival rate of 85.19% at the end of the observation. The survival rate of seagrass seeds in the liquid fertilizer treatment of 3 ml per 100 liters decreased during week 2, with an average survival rate of 96.30% by the end of the observation period. On the other hand, the survival rate of seagrass seeds without the liquid fertilizer treatment decreased during weeks 2 and 3, with an average survival rate of 85.19% at the end of the observation period. The decline in survival value may be attributed to seagrass seeds failure to adapt to the new substrate and environment for seagrass survival. This finding is in line with the perspective presented by Riniatsih *et al*. (2018) study, which indicates that seagrasses usually undergo an adjustment period to aquatic conditions in the first few weeks. Seagrasses must adapt to their new habitat before they can grow normally in the succeeding weeks. The Manova statistical test indicates that liquid fertilizer concentration does not affect the survival rate of seagrass seedlings. The high survival rate is attributed to the rhizome morphology of *E. acoroides*, which provides the capability to endure harsh conditions by strong embedding in the substrate. Moreover, a study by Nugraha *et al*. (2021) reveals that *E. acoroides* species exhibit the highest survival rate among other seagrass species. Additionally, this study controlled environmental conditions better as disruptive factors like currents were absent. Rustam *et al*. (2014) found that currents contribute to transplant failure by washing away the newly transplanted seagrass.

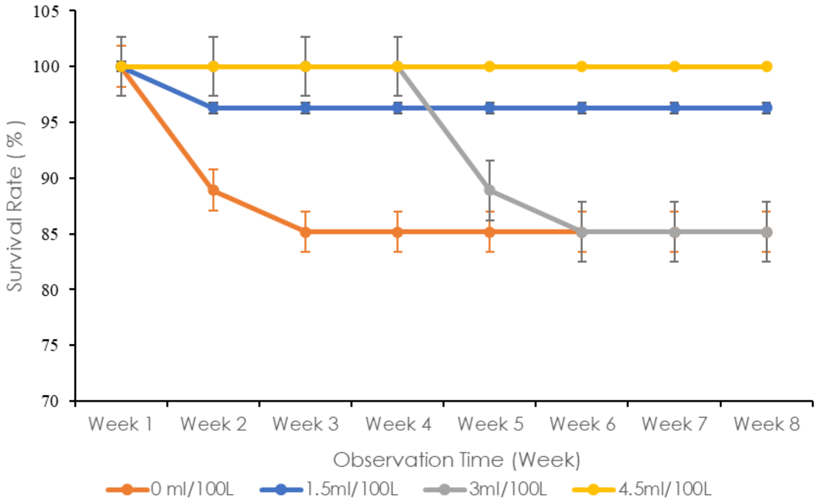

Figure 4. Survival Rate (%) of *E. acoroides* Seed

Over an 8-week observation period, the average daily growth rates of *E. acoroides* seedlings were 0.23 cm/day, 0.20 cm/day, 0.20 cm/day, and 18 cm/day, respectively, for liquid fertilizer concentrations of 4.5 ml/100l, 3 ml/100l, 1.5 ml/100l, and no liquid fertilizer treatment (Figure 6). Artika *et al*. (2020) found that nutrient enrichment at low temperatures impacted the seedlings' growth rate. Additionally, Ravaglioli *et al*. (2017) found that increasing nutrients can increase the growth rate of Posidonia oceanica seagrass by 27%. Nitrogen, when supplied in adequate amounts, can increase the growth of seagrass leaves. Thus, the use of NPK liquid fertilizer, which is a source of nitrogen, can promote the growth of leaf length. The statistical analysis conducted in this study indicates a significant difference in survival rates ($p < 0.05$) for plants treated with liquid fertilizer compared to those that were not. Therefore, it is evident that using liquid fertilizer affects plant survival.

The study demonstrated a positive impact of liquid fertilizer enrichment on the response of *En acoroides* seedlings. However, studies on seedlings of temperate seagrasses have shown no significant positive outcomes of nutrient provision on their physiology, growth, survival, or photosynthetic potential (Alexandre *et al*., 2018). Most research on the effects of nutrient enrichment has been performed on adult seagrasses, yielding mixed results. For instance, Ontoria *et al*. (2019)

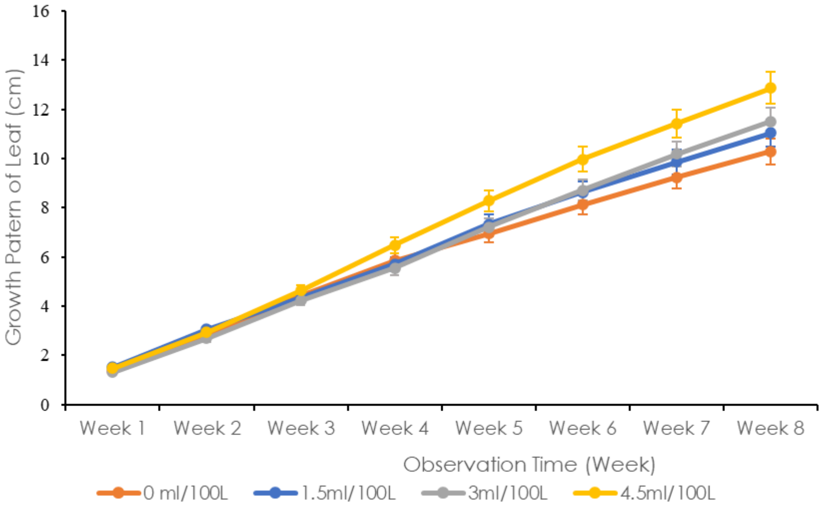

Figure 5. Leaf length growth patern of *E. acoroides* seedlings

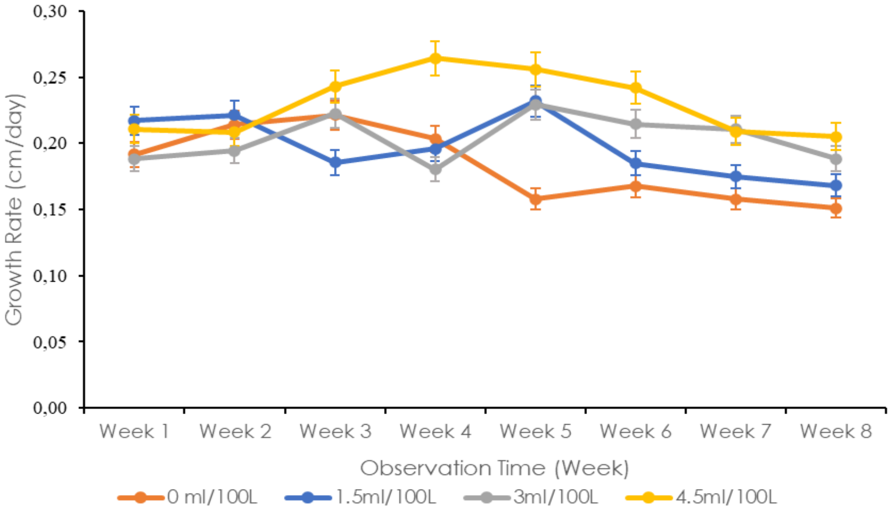

Figure 6. Growth rate of *E. acoroides* seedlings

found that nutrient enrichment tends to impact the nutrient content of seagrass tissues. Still, it may not significantly influence leaf length, leaf width, and seagrass production. Therefore, it can be concluded that initial seedling growth is not solely dependent on nutrition. Although nutrients have positive effects on individual seagrass traits, studies on nutrient enrichment in seagrasses have found indirect negative effects of eutrophication. Nutrient enrichment in nature can cause algal blooms that reduce light and contribute to seagrass decline, as supported by Mahmudi *et al*. (2020). Monitoring the seedling process every week is important. In this study, a water change was performed during the weekly monitoring to reduce attached algae.

The biomass measurement of *E. acoroides* seagrass seedlings in this study is categorized into above ground biomass and below-ground biomass, with the below-ground biomass measurement exceeding the above ground biomass measurement. Aligning with the study by Wahyudi *et al*. (2016), seagrass biomass tends to accumulate at the substrate bottom instead of the top. The rhizome below the substrate contains significantly higher biomass than the biomass above the substrate by 60% to 80%. This is due to the abundance of starch and nutrients found in the seagrass bottom, which are distributed from photosynthesis and preserved in the substrate. A total biomass value ranging from 0.23 to 0.31gram dw/plant was attained from all observation containers, including biomass above and below the substrate (refer to Figure 7) (Supriadi *et al*., 2012). This study is comparable to the research conducted by Artika *et al*. (2020), which found that the total biomass of both the bottom and top substrates of seagrass *E. acoroides* increased with temperature and nutrient treatments. The values obtained ranged from 0.18 to 0.58 gram dw/plant.

The Manova statistical test results indicate that biomass values differ significantly ($p<0.05$). Specifically, the biomass value is higher in the 4ml/100l liquid fertilizer treatment compared to other liquid fertilizer treatments. This result provides evidence that liquid fertilizer treatment impacts seagrass's growth and morphological size, resulting in a higher biomass value. The availability of nutrients, especially phosphate, and nitrogen in the basic substrate, is the dominant factor affecting seagrass growth and its biomass. Phosphate is an essential nutrient that supports seagrass plants' growth and primary production (Qin *et al*., 2021). The average growth rate of seedlings in the 4 ml/100l liquid fertilizer treatment is up to 0.23 cm/day, significantly higher than that of the other treatments. The same applies to the phosphate concentration in seedling container water, which supports growth. In the 4 ml/100l treatment, the phosphate concentration reaches a maximum of 0.07 ppm. The larger size of seedlings grown in the 4 ml/100l treatment could potentially influence the value of seagrass biomass. Research by Graha *et al*. (2016) demonstrates that the biomass value of *E. acoroides* seagrass is higher compared to other types. This indicates that larger morphological sizes lead to greater biomass.

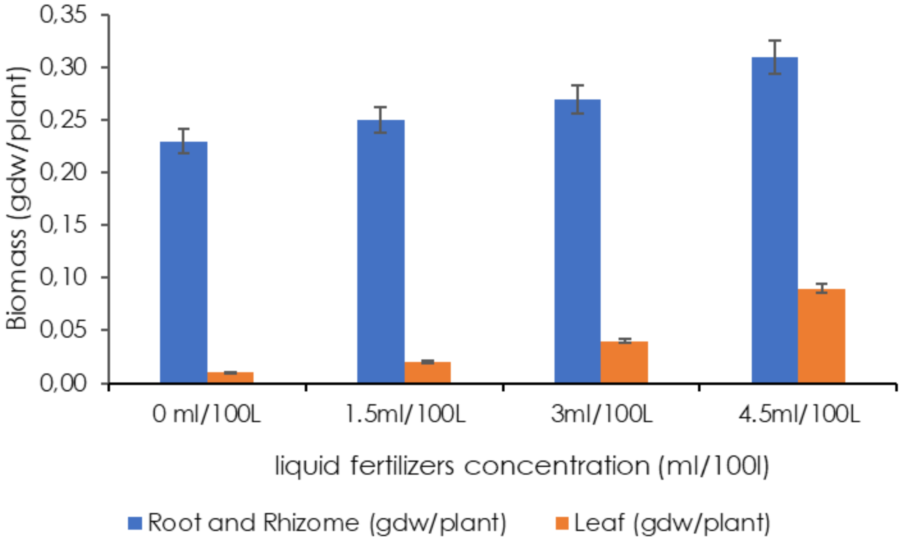

Figure 7. Biomass Value of *E. acoroides* part

Additionally, a higher growth rate positively affects seagrass biomass. The 4ml/100l liquid fertilizer treatment displays a greater growth rate, indicating that seagrass seedlings in this treatment possess good growth and have a higher biomass value. The 4ml/100l liquid fertilizer treatment displays a greater growth rate, indicating that seagrass seedlings in this treatment possess good growth and have a higher biomass value.

Based on the observed results, each chlorophyll-a value of growth rate was obtained from the treatment of liquid fertilizer concentrations of 4.5ml/100l, 3ml/100l, 1.5ml/100l, and without liquid fertilizer treatment, resulting in values of 11.448 mg/g, 7.471 mg/g, 7.130 mg/g, and 5.169 mg/g, respectively (Figure 8). The treatment of liquid fertilizer at a 4 ml/100l rate had the highest chlorophyll-a value. The Manova statistical test revealed significant differences in the chlorophyll-a value ($p < 0.05$), indicating that the liquid fertilizer treatment impacted the chlorophyll-a content present in seagrass leaves. Similar findings were reported by Ravaglioli *et al*. (2017), who found the greatest chlorophyll content in *Posidonia oceana* enriched with high NPK liquid fertilizer under low pH conditions. This indicates that nutrient levels have an impact on chlorophyll concentrations.

N and P elements in liquid fertilizers are considered macronutrients, which subsequently, during the nutrient cycle, are transformed into nitrate compounds and phosphate compounds, respectively (Mustofa, 2015). Macro nutrients like N and P are essential for the growth of plants, including seagrass, and are required in large quantities. Nutrients that impact the growth and development of seagrass include nitrate and phosphate. Lack of nitrate supply can reduce the activity of chlorophyll formation in chloroplasts, leading to a decrease in photosynthesis rate (Subiakto *et al*., 2019). It is essential to note that chloroplasts contain chlorophyll, a pigment that plays a crucial role in photosynthesis. Therefore, the lack of nitrate supply can significantly impact seagrass productivity. Indrawati *et al*. (2013) establish a relationship between leaf chlorophyll-a and nitrate and phosphate in forming chlorophyll, which can enhance photosynthesis and metabolic processes in plants. Ilhami *et al*. (2020) corroborate these findings, noting that nutrients like nitrogen are crucial for creating chlorophyll - a vital green pigment necessary for photosynthesis. Insufficient nutrients, including nitrogen, may hinder chlorophyll formation and impede both growth and photosynthesis in seagrass. However, introducing NPK liquid fertilizer treatment to seagrass seedlings has positively impacted growth, biomass, and chlorophyll-a. Therefore, this technique presents an intriguing topic of discussion for cultivating healthy seagrass seeds and restoring their ecosystem.

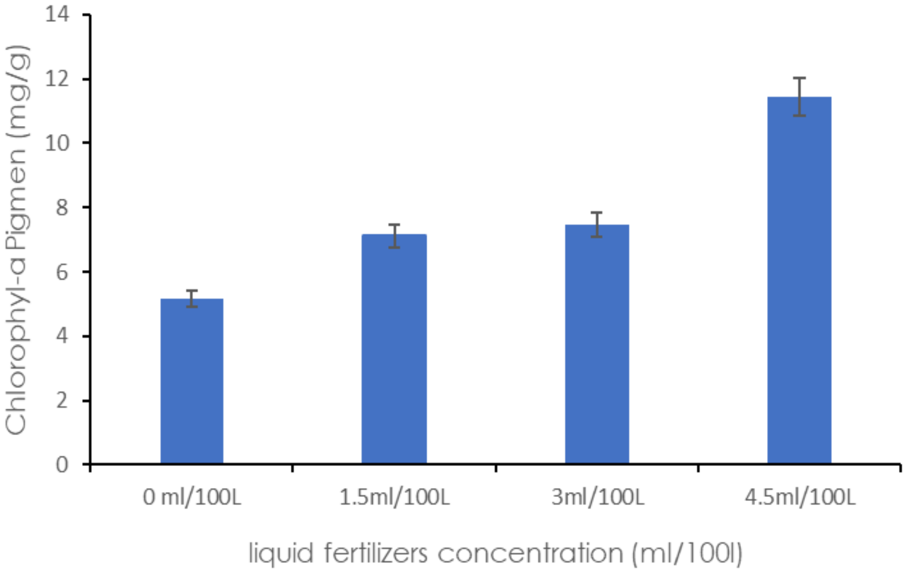

Figure 8. Chlorophyll-a value in seagrass leaves of *E. acoroides*

CONCLUSIONS

Statistical tests demonstrated the impact of NPK liquid fertilizer treatment on the growth, biomass, and chlorophyll-a of seagrass *E. acoroides* seedlings. The research evaluated the response by measuring seagrass leaf growth, survival rate, seagrass stand biomass, and seagrass chlorophyll-a. The greatest increase in leaf growth was observed in the treatment involving 4.5 ml/100l of fertilizer, with an average rate of 0.23 cm/day and a 100% survival rate. The highest seagrass biomass value was found in the 4.5 ml/100 fertilizer treatment, getting an average value of 0.31 gdw/plant in above-ground biomass and 0.09 in below-ground biomass. NPK liquid fertilizer enrichment is a viable approach to ensure adequate seedling availability in the future.

REFERENCES

Alexandre, A., Silva, J., & Santos, R. (2018). Light is more important than nutrient ratios of fertilization for *Cymodocea nodosa* seedling development. *Frontiers in Plant Science, 9*, 768. doi: 10.3389/fpls.2018.00768

Ambo-Rappe, R. (2022). The success of seagrass restoration using *Enhalus acoroides* seeds is correlated with substrate and hydrodynamic conditions. *Journal of Environmental Management*, *310*, 114692. doi: 10.1016/j.jenvman.2022.114692

Ambo-Rappe, R., Nafie, Y.A.L., Syafiuddin, Limbong, S.R., Asriani, N., Handayani, N.T., & Lisdayanti, E.K.A. (2019). Short communication: Restoration of seagrass *Enhalus acoroides using* a combination of generative and vegetative techniques. *Biodiversitas*, *20*(11), 3358–3363. doi: 10.13057/biodiv/d201132

Ambo-Rappe, R., & Yasir, I. (2015). The effect of storage condition on viability of *Enhalus acoroides* seedlings. *Aquatic Botany*, *127*, 57–61. doi: 10.1016/j.aquabot.2015.07.004

Amone-Mabuto, M., Hollander, J., Lugendo, B., Adams, J.B., Bandeira, S. (2022). A field experiment exploring disturbance-and-recovery, and restoration methodology of Zostera capensis to support its role as a coastal protector. *Nordic Journal of Botany,* 2023, e03632. doi: 10.1111/njb.03632

Artika, S.R., Ambo-Rappe, R., Teichberg, M., Moreira-Saporiti, A., & Viana, I.G. (2020). Morphological and physiological responses of *Enhalus acoroides* seedlings under varying temperature and nutrient treatment. *Frontiers in Marine Science*, *7,* 325. doi: 10.3389/fmars.2020.00325

Artika, S.R., Ambo-Rappe, R., Samawi, M.F., Teichberg, M., Moreira-Saporiti, A., Viana, I.G. (2021). Rising temperature is a more important driver than increasing carbon dioxide concentrations in the trait responses of *Enhalus acoroides* seedlings. *Applied Sciences, 11*(6), 2730. doi: 10.3390/app11062730

Burnell, O.W., Russell, B.D., Irving, A.D., & Connell, S.D. (2014). Seagrass response to CO_2 contingent on epiphytic algae: indirect effects can overwhelm direct effects. *Oecologia*, 176(3), 871–882. doi: 10.1007/s00442-014-3054-z

Chamidy, A.N., Suryono, C.A., & Riniatsih, I. (2020). Analisis multivariat untuk melihat hubungan jenis sedimen terhadap jenis lamun. *Journal of Marine Research*, 9(1), 94–98. doi: 10.14710/jmr.v9i1.26686

Dewi, N.K., Kiswardianta, R.B., & Huriawati, F. 2016. Pemanfaatan Serasah Lamun (Seagrass) sebagai Bahan Baku POC (Pupuk Organik Cair). *Proceeding Biology Education Conference*, 13(1), 649-652.

Duarte, C.M., Marbà, N., Gacia, E., Fourqurean, J. W., Beggins, J., Barrón, C., & Apostolaki, E.T. (2010). Seagrass community metabolism: Assessing the carbon sink capacity of seagrass meadows. *Global Biogeochemical Cycles*, *24*(4), 1-8. doi: 10.1029/2010GB003793

Fachrul, M., Haeruman, H., & Sitepu, L.C. (2005). Komunitas Fitoplankton sebagai BioIndikator Kualitas Perairan Teluk Jakarta. Seminar Nasional MIPA 2005. FMIPA Universitas Indonesia, Jakarta.

Fourqurean, J. W., Duarte, C. M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M. A., *et al*. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience, 5*, 505–509. doi: 10.1038/ngeo1477

Graha, Y., Arthana, I.W., & Astawa Karang, I.W.G. (2016). Simpanan karbon padang lamun di kawasan Pantai Sanur, Kota Denpasar. *Ecotrophic : Jurnal Ilmu Lingkungan*, *10*(1), 46. doi: 10.24843/ejes.2016.v10.i01.p08

Granger, S. & Izumi, H. (2001). Water Quality Measurement Methods for Seagrass Habitat. *Journal of Elsevier Science*, 20, 393.

Ilhami, Bq & Mujizat, Kawaroe & Effendi, Hefni & Zamani, Neviaty. (2020). The effect of acidification on growth and photosynthesis rate of seagrass *Thalassia hemprichii (*Ehrenberg.) Ascherson. *Jurnal Ilmu dan Teknologi Kelautan Tropis,* 12(3), 687-696. doi: 10.29244/jitkt.v12i3.24353.

Indrawati A., Hartoko, A. & Soedarsono, P. (2013). Analisa klorofil-a, nitrat dan fosfat pada vegetasi mangrove berdasarkan data lapangan dan data satelit geoeye di Pulau Parang, Kepulauan Karimunjawa. *Journal of Management of Aquatic Resources*, 2(2), 28-37. doi: 10.14710/marj.v2i2.4101

Irawan, A. (2018). Transplantation of *Enhalus acoroides* on a sedimentary beach in Ambon Bay. *IOP Conference Series: Earth and Environmental Science*, 118(1), 012054 doi: 10.1088/1755-1315/118/1/012054

Kawaroe, M., Nugraha, A. H., Juraij, J., & Tasabaramo, I.A. (2016). Seagrass biodiversity at three marine ecoregions of Indonesia: Sunda Shelf, Sulawesi Sea, and Banda Sea. Biodiversitas *Journal of Biological Diversity*, 17(2), 585-591.

Li, H., Liu, J., & Che, X. (2021). Establishing healthy seedlings of *Enhalus acoroides* for the tropical seagrass restoration. *Journal of Environmental Management*, 286, 112200. doi: 10.1016/j.jenvman.2021.112200

Mahmudi, M., Evellin, D.W.L., Endang, Y.H., Lukas, G.S. (2020). Environmental factors and seasonal effect on the potential harmful algae presence at Ambon Bay, Indonesia. *Biodiversitas,* 21(7), 3101-3107. doi: 10.13057/biodiv/d210730

Mustofa, A. (2015). Kandungan nitrat dan pospat sebagai faktor tingkat kesuburan perairan pantai. *Jurnal Disprotek*, 6(1), 13-19. doi: 10.34001/jdpt.v6i1.193

Nugraha, A. H., Almahdi, S., Zahra, A., & Karlina, I. (2021). Morphometric characteristic and growth responses of *Enhalus acoroides* seedlings under different substrate composition treatment. *Omni-Akuatika,* 17(2), 112-117 doi: 10.20884/1.oa.2021.17.1.883

Ort, B.S., Cohen, C.S., Boyer, K.E., Reynolds, L.K., Tam, S.M., & Wyllie-Echeverria, S. (2014). Conservation of eelgrass (*Zostera marina*) genetic diversity in a mesocosm-based restoration experiment. *PLoS ONE*, 9(2), e89316. doi: 10.1371/journal.pone.0089316

Orth, R.J., Lefcheck, J.S., Mcglathery, K.S., Aoki, L., Luckenbach, M.W., & Moore, K.A. 2020. Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services. *Science Advances,* 6(41), eabc6434.

Ontoria, Y., Gonzalez-Guedes, E., Sanmartí, N., Bernardeau-Esteller, J., Ruiz, J. M., Romero, J., & Pérez, M. (2019). Interactive effects of global warming and eutrophication on a fast-growing Mediterranean seagrass. *Marine environmental research,* 145, 27–38. doi: 10.1016/j.marenvres.2019.02.002

Phair, N.L., Toonen, R.J., Knapp, I.S.S., & von der Heyden, S. (2020). Anthropogenic pressures negatively impact genomic diversity of the vulnerable seagrass *Zostera capensis*. *Journal of Environmental Management*, *255*, 109831. doi: 10.1016/j.jenvman.2019.109831

Pratama & Laily. (2015). Analisis Kandungan Klorofil Gandasuli (*Hedychium gardnerianum* Shephard ex Ker-Gawl) Pada Tiga Daerah Perkembangan Daun yang Berbeda. Seminar Nasional Konservasi dan Pemanfaatan Sumber Daya Alam 2015. Pendidikan Biologi, Pendidikan Geografi, Pendidikan Sains, PKLH – FKIP UNS. Universitas Islam Negeri Maulana Malik Ibrahim Malang.

Qin, L.Z., Zhaxi, S., Seung, H.K., & Kun, S.L. (2021) Growth and reproductive responses of the seagrass *Zostera marina* to sediment nutrient enrichment. *ICES Journal of Marine Science*, 78(3), 1160–1173, doi: 10.1093/icesjms/fsab031

Ravaglioli, C., Lauritano, C., Buia, M.C., Balestri, E., Capocchi, A., Fontanini, D., Pardi, G., Tamburello, L., & Procaccini, G. (2017). Nutrient loading fosters seagrass productivity under ocean acidification. *Scientific reports,* 7(1), 13732. doi:10.1038/s41598-017-14075-8

Riniatsih, I., Hartati, R., Endrawati, H., Mahendrajaya, R., Redjeki, S., & Widianingsih, W. (2018). The application of environmental friendly technique for seagrass transplantation. *IOP Conference Series: Earth and Environmental Science*, *116*(1), 012103. doi: 10.1088/1755-1315/116/1/012103

Rustam, A., Bengen, G., Arifin, Z., Lumban Gaol, J., & Arhatin, R.E. (2014). Growth rate and productivity dynamics of *Enhalus acoroides* leaves at the seagrass ecosystem in Pari Islands based on in situ and alos satellite data. *International Journal of Remote Sensing and Earth Sciences*, 10(1), 37-46.

Shinta, Kristiani, & Warisnu, A. (2014). Pengaruh aplikasi pupuk hayati terhadap pertumbuhan dan produktivitas tanaman cabai rawit (*Capsicum frutescens L.*). *Jurnal Sains Dan Seni Pomits*, 2(1), 2337-3520.

Short, F.T., & Duarte, C.M. (2001). Methods for the measurement of seagrass growth and production. *Global Seagrass Research Methods*, pp.155–182. doi: 10.1016/b978-044450891-1/50009-8

Sinclair, E.A., Sherman, C.D.H., Statton, J., Copeland, C., Matthews, A., Waycott, M., van Dijk, K.J., Vergés, A., Kajlich, L., McLeod, I.M., & Kendrick, G.A. (2021). Advances in approaches to seagrass restoration in Australia. *Ecological Management and Restoration*, *22*(1), 10–21. doi: 10.1111/emr.12452

Subiakto, A.Y., Santosa, G.W.,Suryono, S. & Riniatsih, I. (2019). Hubungan kandungan nitrat dan fosfat dalam substrat terhadap kerapatan lamun di Perairan Pantai Prawean, Jepara. *Journal of Marine Research*, 8(1), 55-61. doi: 10.14710/jmr.v8i1.24329

Supriadi, R.F., Kaswadji, Bengen, D.G. & Hutomo, M. (2012). Potensi penyimpanan karbon lamun *Enhalus acoroides* di Pulau Baranglompo Makassar. *Jurnal Ilmu Kelautan*, 19(1), 1 – 10.

Tasabaramo, I.A, Kawaroe, M. & Rappe, R.A. (2015). Laju pertumbuhan, penutupan dan tingkat kelangsungan hidup *Enhalus acoroides* yang ditransplantasi secara monospesies dan multispesies. *Jurnal Ilmu Dan Teknologi Kelautan Tropis*, *7*(2), 757-770

Van Katwijk, M.M., Bos, A.R., de Jonge, V.N., Hanssen, L.S.A.M., Hermus, D.C.R., & de Jong, D.J. (2009). Guidelines for seagrass restoration: Importance of habitat selection and donor population, spreading of risks, and ecosystem engineering effects. *Marine Pollution Bulletin*, *58*(2), 179–188. doi: 10.1016/j.marpolbul.2008.09.028

Vieira, V.M.N.C.S., Lobo-Arteaga, J., Santos, R., Leitão-Silva, D., Veronez, A., Neves, J.M., Nogueira, M., Creed, J.C., Bertelli, C.M., Samper-Villarreal, J. & Pettersen, M.R.S. (2022) Seagrasses benefit from mild anthropogenic nutrient additions. Frontiers in Marine Science, 9, 960249. doi: 10.3389/fmars.2022.960249

Nielsen, O.I., Koch, M.S., Jensen, H.S., Madden, C.J. (2006). Thalassia testudinum phosphate uptake kinetics at low in situ concentrations using a IT radioisotope technique. *Limnology and Oceanography,* 51, 208-217. doi: 10.4319/lo.2006.51.1.0208

Wahyudi, A.J., Rahmawati, S., Prayudha, B., Iskandar, M.R., & Arfianti, T. (2016). Vertical carbon flux of marine snow in *Enhalus acoroides* dominated seagrass meadows. *Regional Studies in Marine Science*, *5*, 27–34. doi: 10.1016/j.rsma.2016.01.003

Wagey, B.T. 2013. Variation in Chlorophyll a and b in the Seagrass Halodule in Central. *IAMURE Multidisciplinary Research*, 8(1), 131-163.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences of the United States of America,* 106, 12377–12381. doi: 10.1073/pnas.0905620106.

Wismar, J.E., Ambariyanto, & Widianingsih. (2023). Seagrass (*Enhalus acoroides*) restoration performance with two different methods (Anchor and Seed) in Panjang Island, Jepara, Indonesia. *Jurnal Ilmiah Perikanan Dan Kelautan*, *15*(1), 84–94. doi: 10.20473/jipk.v15i1.35836

Weitzman, J.S., Zeller, R.B., Thomas, F.I.M., & Koseff, J.R. (2015). The attenuation of current- and wave-driven flow within submerged multispecific vegetative canopies. *Limnology and Oceanography*, 60(6), 1855–1874. doi: 10.1002/lno.10121

Yu, S., Wu, Y., Serrao, E.A., Zhang, J., Jiang, Z., Huang, C., Cui, L., Thorhaug, A., & Huang, X. (2019). Fine-scale genetic structure and flowering output of the seagrass *Enhalus acoroides* undergoing disturbance. *Ecology and Evolution*, 9(9), 5186–5195. doi: 10.1002/ece3.5106

Zamani, N.P., Prartono, T., Arman, A., Ariesta, D.S., & Wahab, I. (2018). Concentration of heavy metals on roots, stem and leaves of *Enhalus acoroides*, in Tunda Island, Banten Bay. *Jurnal Ilmu Dan Teknologi Kelautan Tropis*, *10*(3), 769–784. doi: 10.29244/jitkt.v10i3.23172

Zhang, Y.H., Li, C., Zhao, J. S., Li, W.T., & Zhang, P.D. (2020). Seagrass resilience: Where and how to collect donor plants for the ecological restoration of eelgrass *Zostera marina* in Rongcheng Bay, Shandong Peninsula, China. *Ecological Engineering*, *158,* 106029. doi: 10.1016/j.ecoleng. 2020.106029