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Research Article

Seagrass (*Enhalus acoroides*) Restoration Performance with Two Different Methods (Anchor and Seed) in Panjang Island, Jepara, Indonesia

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Abstract

Globally, the area of seagrass beds important to the ecosystems in coastal environment is decreasing due to environmental pressures, both natural and artificial. Transplantation is one way to restore the condition of damaged seagrass beds, the anchor transplant method uses the transfer of donor seagrass to the transplant area, while the seed transplant method uses seed sowed from seagrass. This study aimed to investigate the survival and growth rates of seagrass transplants and the biomass and chlorophyll content of transplanted *Enhalus acoroides* in the waters of Panjang Island Jepara utilizing anchor and seedling methods. The research was conducted in the waters around Panjang Island, Jepara, between September and December 2021. The results indicated that transplantation of seagrass *E. acoroides* using the anchor method resulted in the highest average growth rate of 0.25 cm/day and a survival rate of 96.67%, while transplantation using the seedling method resulted in a growth rate of 0.18 cm/day and a survival rate of 83.33%. The results showed that the anchor method scored better than the seedling transplantation method in terms of growth rate and survival rate. However, seedling is a feasible method to meet the availability of seeds that will later be transplanted, considering that the anchor method still has shortcomings in terms of the availability of seeds and the use of pegs which are still not environmentally friendly. So that the seedling method can be recommended for the restoration process of seagrass ecosystems by taking into account the season, weather and other important indices.

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1. Introduction

Seagrass beds worldwide are currently experiencing a decline in cover conditions. Since the 2000s, about 29% of seagrass ecosystems in the world have disappeared, making the seagrass ecosystem one of the most threatened ecosystems in the world (Wendländer *et al.*, 2019). In Indonesia, about 40 - 60% of seagrass beds in Indonesian coastal waters have been damaged and their area reduced, this is due to human activities (Sjafrie *et al.*, 2018). For example, on Pari Island, Jakarta, the seagrass area decreased by 678,300 m^2 or around 25% over five years (1999-2004) (Rahmawati, 2011), and 5,700 m^2 in Bontang coastal areas (Irawan *et al.*, 2019), and 229.000 m^2 in Banten waters (Sugianti and Mujiyanto, 2020). Seagrass conditions in Indonesia, have an average seagrass cover of 39 ± 3.6% (mean ± SE) which is lower than that measured 3 years earlier (46%). The data shows that the seagrass beds in Indonesia are generally in moderate condition, indicated by an average SEQI value of 0.68 ± 0.02. These findings provide a basis for seagrass conservation and management (Hernawan *et al.*, 2021).

Seagrass beds play a critical role in shallow marine ecosystems, including buffer zone for fish biodiversity, and as such, efforts to protect and proper management of seagrass ecosystems are necessary (Hartati *et al.*, 2017; Irawan *et al.*, 2018; Tebaiy *et al.*, 2021). The true impact of seagrass deterioration is a decrease in the diversity of marine biota due to the seagrass ecosystem's fall in ecological function (Tangke, 2010; Riniatsih *et al.*, 2021). Restoration is necessary to restore this function. Seagrass restoration activities have been conducted using various methods with varying degrees of success (Azkab, 2000), as Kawaroe *et al.* (2008) did by transplanting seagrass species, *Enhalus acoroides,* and *Thalassia hemprichii*, in Seribu Islands using a variety of methods. Kiswara (2004) also transplanted seagrass in Banten Bay between 1999-2001, utilizing the cracked shoot planting technique.

Restoration can be accomplished by using spring-anchored or non-anchored transplantation methods, namely turfs, plugs, and seedlings which is a germination process in an effort to provide transplant seeds. The anchor, turf, and plug methods involve the removal of one or more mature plant stands from the seagrass area and replanting them in areas that have suffered damage or decreased seagrass bed conditions (Wulandari *et al.*, 2013). In contrast, the seedling method involves using seeds collected from old fruit and then sown to become seagrasses that are ready to be planted in the field (Thorhaug, 1974). Many researchers in Indonesia have used this transplant procedure. From 1999 to 2001, Kiswara (2004) used anchor and non-anchor techniques on the seagrass *Enhalus acoroides* in Banten Bay, with survival rates varying from 60% to 80%. Kawaroe *et al.* (2008) also carried out the transplantation of the plug, frame method with a 100% success rate in the Thousand Islands, and research by Rosmawati *et al.* (2020) achieved a 100% transplant success rate in the waters of Waai Village.

The anchor method is a method of transplantation in which plant seeds are collected from donor sites and affixed to anchors that are then put into the substrate until the seagrass roots are completely buried (Short and Coles, 2001). Anchors might be in the shape of staples, rods, or rings. The procedure has disadvantages if the seed donor location is located a considerable distance from the transplant site (Riniatsih and Endrawati, 2013). Anchors are utilized in both methods in locations with currents or bumpy areas due to the wind to prevent plants from being blown away by the current (Azkab, 2000).

The seedling method is an alternative to overcome the problem if the donor location is distant from the transplant location. Seagrass seeds and fruit are collected from the donor location and placed in media for sowing after determining the appropriate seedling age (Lewis *et al.*, 1982). Seedling maintenance aims to maintain the condition of the seagrass so that it can adapt to a supportive environment and reach the size of the shoot, at which point planting/transplanting can be done (Ort *et al.*, 2014). The seedling method was initially used in calm water with few predators in large-scale restoration. Thorhaug (1974) used this procedure to plant or transplant *Thalassia testudinum* seeds. Azkab (2000) reported that Thorhaug (1974) employed *Thalassia testudinum* seeds in their studies. Direct sowing from collected seeds has been attempted on a wide scale in Biscayne Bay and Florida's small islands. However, it has proved unsuccessful due to poor water conditions (strong currents and waves) that cause planted seeds to be uprooted from the substrate. Research conducted by Ambo-Rappe (2022) stated that the restoration of seagrass using seeds is a feasible method because of the high availability of seeds. The data from the seedling study showed that an average of 64% of the scattered seeds could develop and survive until the 40th day of observation. One way to improve the success rate of seedling transplantation is to sow it in a more controlled and protected environment and then replant it in nature at a certain age. Planting is done by a method that is adapted to the conditions of the transplant waters, such as the addition of anchors. Pre-planting seeding is done to enable them to defend themselves against predators and extreme environments (Borum *et al*., 2004).

According to Kilminster *et al.* (2015), *E. acoroides* is classified as a persistent seagrass, which means that it can adapt to more extreme environmental conditions than other forms of seagrass most effectively in seagrass transplantation water. Combining the two methods of seagrass rehabilitation (vegetative and generative) is thought to increase the survival of seagrass seedlings, because adult seagrasses (anchor method) can act as an important and effective protection for seedlings (seedling method), reducing substrate movement and reducing the strength of wave action.

This research was conducted at Panjang Island, Jepara, which has a great diversity of seagrass species and favorable seagrass environments (Pradhana *et al*., 2021). Panjang Island waters is one of the small islands in Jepara Regency, Central Java Province. This island is located directly at the mouth/front of Awur Bay's waters (Kusuma *et al*., 2020) and is home to coral reef ecosystems, sea pine forests, and seagrass beds. Enhalus *acoroides* dominate the seagrass ecosystem on the eastern side of the island. Panjang Island's seagrass beds cover 76.75% and are in excellent health. Many activities on Panjang Island include tourism, fishing, and ship-related activities (PLTU Tanjung Jati B, 2020). The purpose of the current study is to determine the success of seagrass species *Enhalus acoroides* transplantation by comparing the growth and survival rates of seagrass using the spring anchored and seedling method in the waters of Panjang Island, Jepara, Central Java.

2. Materials and Methods

2.1 Research Materials

The research material used in this study was seagrass (*Enhalus acoroides*), which was obtained from donor sites as tissue (roots, stems, leaves, fruit, and seeds).

2.2 Research Location and Sites

The research area was determined using the purposive method, which selects the research area based on information and facts about the research area (Singarimbun and Effendi, 1995). The primary requirement for this study is the presence of a seagrass ecosystem in the waters surrounding Panjang Island, Jepara.

The seagrass transplant was conducted on a 50 cm x 50 cm transplant plot randomly divided into three plots (anchor, seedling, and natural vegetation plot as control). The location was determined based on the area, which had a history of being overgrown with seagrass but was damaged due to physical, chemical, and biological factors (Riniatsih and Endrawati, 2013). The research area was chosen based on the density of seagrass, cover, and water parameters considered indicative by the PTSI index (Preliminary Transplant Suitability Index) (Short *et al*., 2002). The research area on Panjang Island, Jepara, with blue dots representing monitoring stations and red dots representing transplant stations (TA: natural vegetation; TJ: anchor; and TS: seedling) (Figure 1).

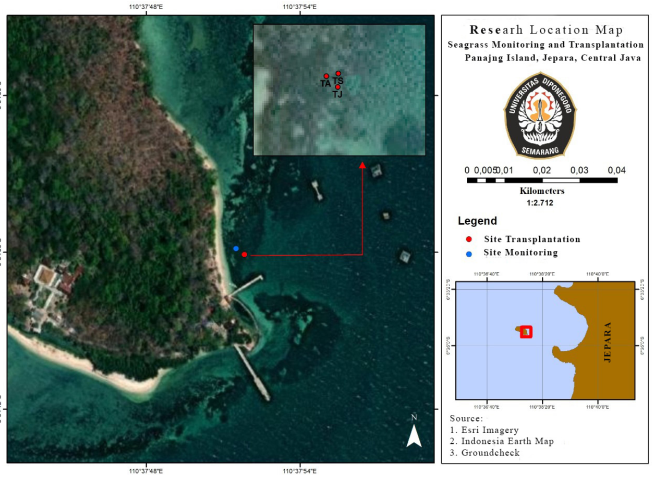

Figure 1. Map of research sites on Panjang Island, Jepara, Central Java (TA: Natural Vegetation; TJ: Anchor; and TS: Seedling).

Various data on the existing seagrass beds (reference sites) and the location of seagrass transplants (recipient sites) are needed to calculate the Preliminary Transplant Suitability Index (PTSI. At each location, the results of environmental parameter measurements were tabulated and assigned a score. The values 0, 1, and 2 denote the quality of the measured parameter. A value of 1 means that the location is supportive and a value of 2 means that the location is very supportive for seagrass transplants. The PTSI scores for all parameters are summed. A value of 0 for several parameters results in a score of 0 and removes that place from the priority list (Short *et al*., 2002). A high score suggests a good chance of successfully transplanting seagrass (BTNKpS, 2006).

The Preliminary Transplant Suitability Index (PTSI) parameters consist of seagrass presence, distance from existing seagrass beds, water clarity, primary particle size, depth, sediment, salinity, temperature, pH, and current/wave.

The observation plot on seagrass transplants will be separated into different treatment methods. The selection of planting site by examining the water conditions (depth, brightness, and substrate) are the same (Figure 2).

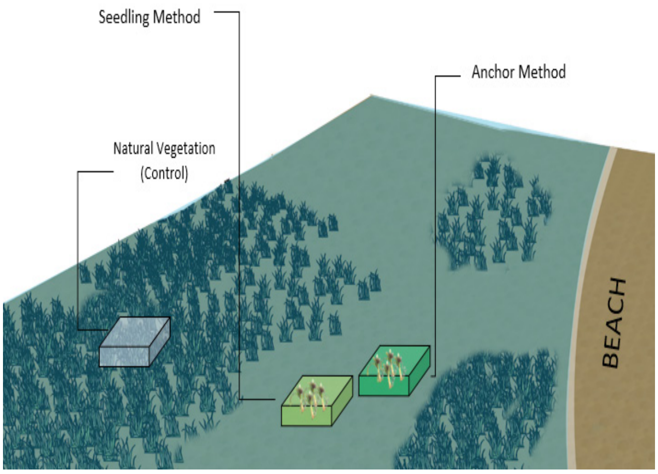

Figure 2. Configuration of the transplant observation plots

2.3 Collecting Seagrass Seeds for Anchor Method Transplant

Enhalus acoroides was chosen for this study due to its widespread distribution and the availability of healthy seeds (Harnianti *et al*., 2017). Seagrasses that shoot fresh have white rhizome characteristics, and at least two new shoots are needed for seed collection (Ambo-Rappe and Yasir, 2015). *E. acoroides* is a persistent species that grows abundantly in open tidal environments and is the main species distributed over the transplant site (Kilminster *et al*., 2015). The type of *E. acoroides* must have a 15 cm long rhizome, leaves 30 cm in length, and a growth point (meristem) at the rhizome's tip (Tasabaramo *et al*., 2016). Using a crowbar or shovel, 25 seeds of *E. acoroides* were collected. The prepared crowbars were stabbed into the substrate around the seagrass seedlings to create a space to reach the roots. Leaves, rhizomes (stems), and roots were collected as individual seeds which will later be planted in the field. The seagrass seeds were then selected to obtain healthy seeds that are ready to be stored in containers filled with saltwater and away from direct sunlight to prevent the seagrass seeds from wilting.

2.4 Seedling Process in Laboratory

Mature (fruiting) seagrasses *E. acoroides* were selected in donor waters. Ripe fruit was identified by a portion of the fruit stem that feels solid in the hand and hair on the fruit that becomes shorter and less stiff. The fruit of the seagrass was plucked on a stalk around 5 cm from the fruit. Seagrass fruits and seeds were then placed in bags for transfer to the seedling container (Thorhaug, 1974). Next, they were selected to get a uniform size for sowing in prepared containers. The fruit of *E. acoroides* was opened, and the seeds were removed carefully so as not to damage the seed coat. A total of 20 fruits are collected to produce 40 seeds of *E. acoroides* and placed in polybags with a substrate. They were randomly placed in seeding containers filled with seawater and connected via a recirculation system (Figure 3). Five weeks before planting/transplantation, the seeds and fruit were sown (Hasanah, 2014). Each polybag is labeled for growth measurement on each seed sown. Seagrass seedling growth was measured by counting the number of leaves, growth rate, and survival rate (Ambo-Rappe and Yasir, 2015). Improvement of seed quality is made by adding NASA fertilizer (Ilhami, 2019) into the water where the seeds are stored so that the seeds still receive sufficient nutrients and are ready for transplantation.

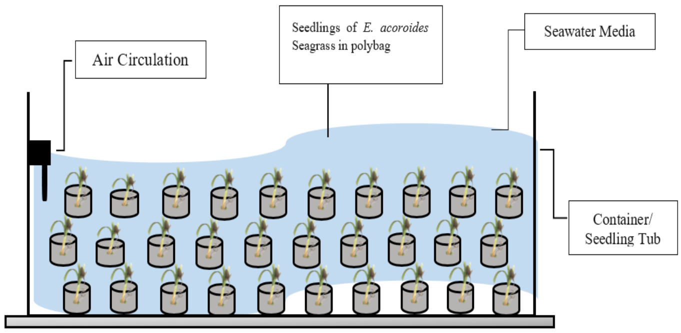

Figure 3. Seed Germination process of the seagrass seeds in the container

2.5 Transplant Process

Three cages or frames for anchor, seedling, and natural vegetation were constructed from transplant plots using nets that surrounded the plot area of 50x50 cm. These cages were used to ensure that seagrass transplants in the field were not disturbed by human activities, grazers, or natural conditions (Figure 4).

Seagrass seeds that had been prepared (pre-sorted) were then tied to 30 cm bamboo stakes using mattress threads to avoid plastic waste during the transplantation procedure. Pegs were employed during the seedling planting process to prevent the seagrass seedlings from being uprooted by water currents or waves. In the planting area, holes were drilled with the corer/crowbar to insert or plug the prepared seeds (Grech *et al.*, 2012). The process of planting the seagrass was aligned with the position of the transplant plots using the anchor and seedling method plot, which was filled with 25 ready-to-plant seagrass seedlings (Figure 4).

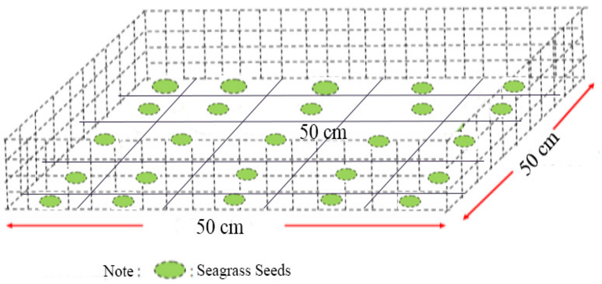

Figure 4. Seagrass transplant plot frames

The numbering of transplant units is performed to allow for additional observations. Transplant units are placed in rehabilitation locations based on poor locations identified through seagrass vegetation analysis.

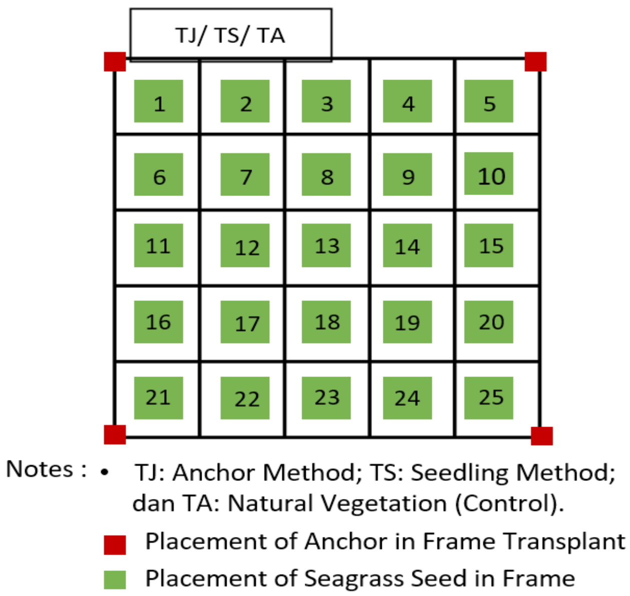

Figure 5. Placement of seagrass seedlings on the transplantation frame using the anchor to seedling method and marking on each seagrass seed

2.6 Growth and Survival of Seed

The success of the transplant was determined by the growth rate and condition of the transplant unit. The rate of seagrass leaf growth was determined by quantifying the absolute leaf growth. Observations on transplantation success and the growth rate of seagrass leaves were performed on each plot (Figure 5). The observation processes were conducted every two weeks for three months (12 weeks) since planting (Riniatsih and Endrawati, 2013). Natural vegetation was used as a control for determining growth rate by placing the same plot in the location of natural seagrass vegetation. The seagrass selected for this study must have a size that is not significantly different from the size of the seagrass seedlings in the anchor, seedling, and natural vegetation plot. Additionally, water condition factors such as temperature, salinity, pH, brightness, dissolved oxygen, and the presence or absence of sedimentation and currents were measured (Short *et al.,* 2002). The leaf marking method, developed by Sand-Jensen (1975) and Azkab (2000), was used to determine the growth rate of seagrass leaves. The seagrass marking methods are based on marking/perforating leaves or stands at a certain frequency. Additionally, measurements of seagrass biomass, chlorophyll, and nitrate phosphate were performed to acquire a full view of the transplanted seagrass's growth.

Growth rate, calculated using the equation (Short and Coles, 2001):

$$P = \frac{(Pt - P0)}{t}$$

Where:

P = Growth rate (cm/day)

Pt = final length (cm)

P0 = initial length (cm)

t = observation time (day)

2.7 Data Analysis

The results were analyzed descriptively. However, a one-way analysis of variance (ANOVA) was applied to compare the growth and survival of seagrass seedlings to the transplantation method used.

3. Results and Discussion

3.1 Preliminary Transplant Suitability Index (PTSI) *Assessment*

The research area coordinates are 6°34'35.0"S 110°37'51.6"E, where a PTSI assessment was conducted first to determine the quality of each parameter assessed and whether the area was possible for seagrass transplantation (Short *et al*., 2002).

Table 1. PTSI (Preliminary Transplant Suitability Index) of seagrass transplant area

No	Parameters	Score
1	Seagrass presence	2 (Two)
2	Distance from existing seagrass beds	2 (Two)
3	Water clarity	2 (Two)
4	Primary particle size	2 (Two)
5	Depth	2 (Two)
6	Sediment	2 (Two)
7	Salinity	2 (Two)
8	Temperature	2 (Two)
9	pH	2 (Two)
10	Current/Wave	2 (Two)
	Total	**20 (Twenty)**

The results of PTSI analysis show that the area selected was excellent and ideal, as the environmental characteristics evaluated were extremely favorable for the seagrass transplantation process (Table 1). A score of 20 indicates that the place has a high probability of success in the transplant process compared to seagrass beds' quality standards (BTNKpS, 2006). Location suitability is determined by the average value of each criterion, which represents the maximum value of 2 in the assessment. The parameters' PTSI values indicated that the chosen location is appropriate and suitable because the parameters measured are in the optimal range and meet water quality standards, particularly for *E. acoroides*.

3.2 Growth Rate of Enhalus acoroides

The results indicated that the average growth rate of seagrass *E. acoroides* transplanted using the anchor, seedling methods, and control were 0.25, 0.18, and 0.31 cm/day, respectively (Figure 6). The difference in values between the natural vegetation plot and other methods is due to the fact that the seagrass in the natural vegetation plot does not need to adapt to environmental changes (substrate and water parameters), while the seagrass transplanted using the seedling method does (lowest growth rate values). This is consistent with Thangaradjou and Kannan (2008), who claimed that seagrass requires an adaptation period to its new habitat before growing normally in the early weeks. Hasanah (2014) also stated that old leaves grow slower than young leaves, which means that the seedling method had the lowest growth rate value, however, it had good growth. The seedling transplantation method needs to be maintained and considered as an alternative transplant method because seagrass restoration using seeds has been recommended to preserve genetic diversity and minimize damage to donor sites (Ambo-Rappe, 2022).

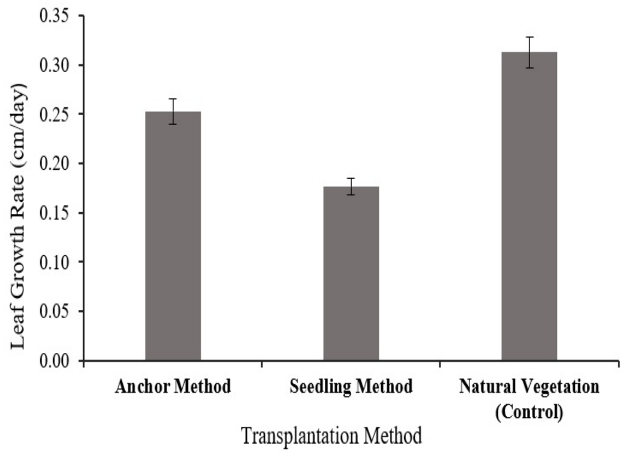

Figure 6. Average growth rate (±SD) of seagrass *E. acoroides*

The growth rates of the anchor method, seedling method, and natural vegetation (control) were statistically significant (P= 0.012), indicating that the differences in transplantation methods significantly affect seagrass growth rate. The average growth rate of seagrass *E. acoroides* transplanted in the waters of Panjang Island, Jepara, was lower than that found by Supriadi *et al*. (2006). They found that the growth rate of seagrass *E. acoroides* in Barrang Lompo Island was 1.20 cm/day. In addition, Rustam *et al*. (2014) found that the growth rate of seagrass *E. acoroides* on Pari Island during the transitional season was 3.9-5.6 cm/day. The slow growth rate of transplanted seagrass *E. acoroides* is due to the adaptation process to a new environment with different environmental conditions, most notably substrate conditions, where the donor site has a muddy substrate. In contrast, the transplant site has a sand substrate and a muddy sand substrate (Kiswara, 2004). The different conditions of seagrass in each location may be influenced by differences in leaf production related to differences in leaf morphology, and conditions of nutrient concentration (water column and sediment) between locations (Sumbayak *et al*., 2021). However, when compared to a study conducted by Talakua and Rumengan (2020), with a growth rate of seagrass *E. acoroides* of

0.23 cm/day using the anchor method, and a study conducted by Mustaromin *et al.* (2019), with a growth rate of 0.23-0.65 cm/day in the TERFs transplant method, and in a study conducted by Hasanah (2014), with a value of 1.72-1.94 mm/day the results of the current study were not much different and even tended to show slightly higher growth rate. These results were probably due to the artificial barrier that acts as a barrier to currents and waves, thereby protecting the transplanted seagrass seedlings. Ambo-Rappe *et al.* (2019) stated that it is very important to protect the transplanted seedlings from waves and stockpiling substrate, which can lead to poor seagrass conditions. Seagrass growth can be influenced by environmental factors such as temperature and pH (Supriadi *et al.*, 2006; Nugraha *et al.*, 2021). If the temperature and pH parameters are not in accordance with their optimal numbers, they can affect seagrass life, such as activating life, respiration, photosynthesis, nutrient absorption, growth and morphometric characteristics (Supriadi *et al.*, 2006; Nugraha *et al.*, 2021).

The growth pattern on natural vegetation plots appears to be distinct from the anchor and seedling transplantation methods. The natural vegetation plot has a decrease in growth rate at week 6, then increased again at week eight and dramatically decreased again at week 10, before increasing again at week 12 to the end of the observation (Figure 7). The same pattern occurred with the anchor and seedling methods (maturity). Similarly, at week 10, all transplantation methods and natural vegetation plots had a simultaneous reduction, presumably because weeks 9 to 10 (mid to late October) marked the start of the season entry period. In the west, where the intensity of rainfall is high and accompanied by strong winds resulting in strong waves and currents that cause the water to become very murky (10 cm brightness) (Ilahude and Nontji, 1999). Turbid water can be caused by sediment or substrate that is lifted in each plot so that it affects the growth of *E. acoroides* seagrass. Turbid water can be detrimental to seagrass because sunlight cannot be used to its full potential during photosynthesis (Wulandari *et al.*, 2013).

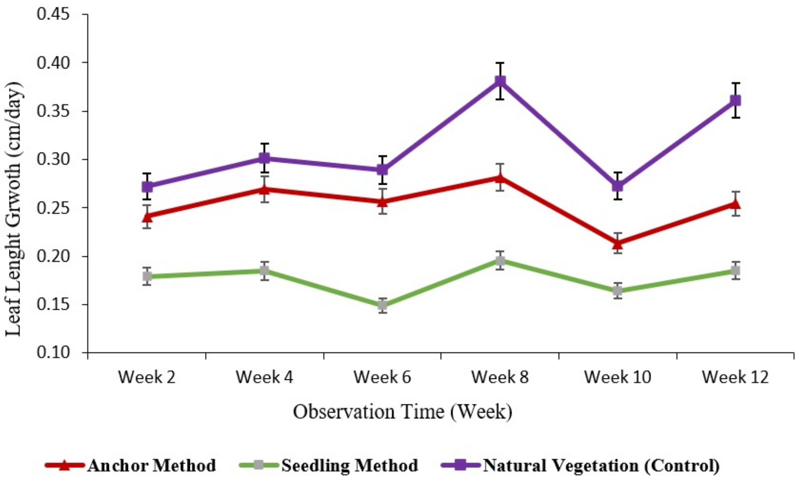

Figure 7. Growth pattern of seagrass *Enhalus acoroides* leaves

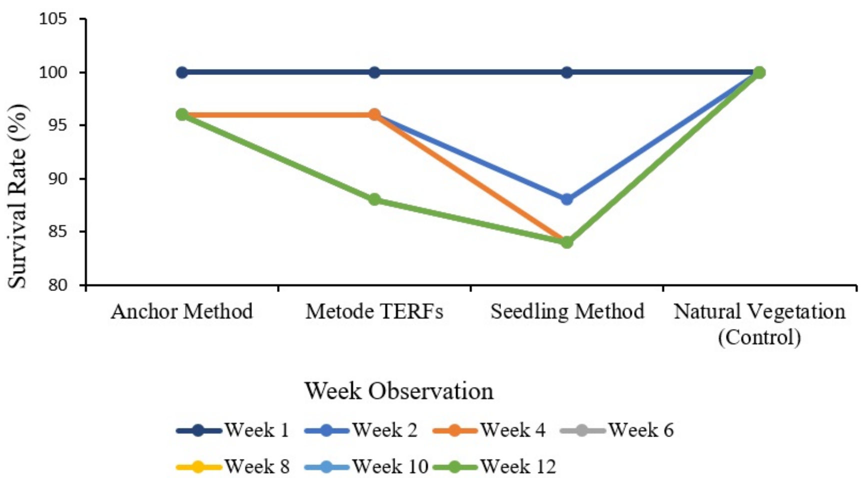

Figure 8. Survival rate of seagrass transplant *E. acoroides*

3.3 Survival Rate of Enhalus acoroides

The survival rate was observed every two weeks for 12 weeks and decreased with each week, except for natural seagrass plots (natural vegetation/control), which had a 100% survival rate from the start to the end of the observation. During the first week of observation, specifically the second week, it was observed that the survival rates for the anchor and seedling methods decreased. The anchor method's survival rate decreased to 96%, while the seedling method decreased to 84%. However, the survival rates for the natural seagrass plots remained constant (Figure 8).

The highest average survival rate of *E. acoroides* was obtained in natural vegetation plots (control) at 100%. This result is because natural seagrasses exhibit greater resistance and growth than transplanted seagrasses (Calumpong and Fonseca, 2001). Anchors also have a higher survival rate than seedling methods, i.e., 96% and 84%, respectively. The reduction happens on average between two and four weeks. The decrease in survival rate is because transplant units cannot adjust to their new substrate and environment (Lanuru, 2011). In addition, *E. acoroides* transplanted with the anchor method developed roots that measure 7.70-27.10 cm in length, allowing them to be firmly attached to the substrate. Additionally, the anchor method used in the transplant unit significantly affects the adaptation of the rhizome of *E. acoroides* to the substrate at the transplant site (Harah and Sidik, 2013). Several results of transplant research using the anchor method have also demonstrated a reasonably high success rate. Lanuru (2011) observed that the survival rate of *E. acoroides* transplanted in the Labakkang area for one month was between 95% and 100%. In addition, Talakua and Rumengan (2020) reported a similar result with a survival rate of 90% using the anchor method.

The seedling method is the method with the lowest survival rate of 84% with the death of 4 seagrass seedlings where the decline occurs at week 2 and week 4. The decrease occurred allegedly because in week 4 the observations were made when the environmental conditions of the waters were not good enough, namely when the west wind season occurs which made the currents and waves quite large (current speed ranges from 0.7 m/s to 0.9 m/s in the northwest direction). The factor of waves and large currents due to the west monsoon can make the bottom sediment transported and hit the transplant unit or uproot the transplant unit from the pegs used. Seagrass seedling mortality can also be caused by stress due to different salinity conditions between seeding aquariums with a salinity of 28-33 ppt and seawater at the transplant site with a salinity value of 31-33. The optimum value that can be tolerated by seagrass plants is 30-35 ppt (PP No.22 of 2021), the tolerance of seagrass to salinity varies with the type and age of seagrass. Old seagrasses can tolerate large salinity fluctuations, while young seagrasses can be damaged because low salinity will reduce the ability of seagrasses to carry out photosynthesis (Dahuri *et al*., 2001). Lanuru (2011) stated that sediment stability is critical for keeping the sediment and transplant unit in place and not carried away by strong waves and currents. Seagrass seedlings sown in the laboratory have thinner and smaller leaves than natural seagrass seedlings, owing to the natural sea providing nutrients compared to artificially produced seedling containers. The survival rate of seagrass *E. acoroides* in the seedling method was quite good in comparison to the research by Thangaradjou and Kannan (2008), which found that the same method achieved a survival rate of 29% in the seagrass species *Ruppia maritime*, and was not significantly different with the same method done by Hasanah (2014) at Baranglompo Island, i.e., 94%.

The survival rate values for the three transplantation methods (anchor, seedling, and natural vegetation plots) were statistically different (P= 0.016). Each transplantation method has a different approach to the planting process and also has a different pattern of adaptation to growth, including how seeds are planted into the substrate. The ability of the transplant method to withstand exposure to currents or waves is the primary factor (Rinatsih and Endrawati, 2013), so seagrass adaptation failure is very likely to occur during the 12 weeks of observation (Wulandari *et al*., 2013).

4. Conclusion

The growth rate of seagrass *Enhalus acoroides* transplanted using the anchor, seedling, and natural vegetation was 0.25, 0.23, and 0.18 cm/day, respectively. Overall, each method had a survival rate of 96% for the anchor method and 84% for the seedling method. Indicating that the anchor method is better than the seedling transplantation method in terms of growth rate and survival rate. However, seedling is a feasible method to meet the availability of seeds that will later be transplanted. considering that the anchor method still has shortcomings in terms of the availability of seeds and the use of pegs which are still not environmentally friendly.

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Authors' Contributions

All authors have contributed to the final manuscript.

Conflict of Interests

The authors declare that they have no competing interests.

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